



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

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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.

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); prothionamide (Pto); cycloserine (Cs); terizidone (Trd); <i>p</i> -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.

Aminoglycosides, polypeptides and fluoroquinolones are bactericidal (able to achieve killing of mycobacteria), while thioamides, cycloserine/terizidone and para-aminosalicylic acid (PAS) are bacteriostatic (able to prevent growth of mycobacteria).

Once the injectable drugs and the fluoroquinolones are compromised by resistance, available treatment regimens become much weaker and the possibility for patient cure decreases significantly. Significantly more clinical data are needed to answer key questions relating to treatment outcomes in the presence of different combinations and permutations of drug resistance. However, evidence from Latvia shows that the rate of successful treatment outcome precipitously falls as resistance to the key SLDs increases – in a cohort of 820 MDR-TB patients who had completed treatment with

SLDs, two thirds overall had a successful outcome. However, in those patients with strains resistant to an injectable drug (kanamycin or capreomycin) and a fluoroquinolone (ofloxacin), less than 30% were successfully treated. Among those with strains resistant to kanamycin and ofloxacin specifically, only 24% had a successful treatment outcome (personal communication, Vaira Leimane, Latvia; Timothy Holtz, CDC).

Where bacterial resistance to aminoglycosides, polypeptides and fluoroquinolones is associated with additional resistance to the bacteriostatic drugs, treatment regimens are weakened even further and become virtually ineffective. As has been shown in South Africa (3), such infections are lethal for HIV-coinfected patients.

Reliability and reproducibility of second-line DST

Aminoglycosides, polypeptides and fluoroquinolones have been tested in different laboratory environments and shown to have relatively good reliability and reproducibility (6–8, 11–13). Data on the reproducibility and reliability of DST for the other SLDs are either much more limited or have not been established, or the methodology for testing does not exist.

Most importantly, correlation of second-line DST results with clinical response to treatment has not yet been adequately established. As a result, the prognostic relevance of in vitro resistance remains unclear for the majority of SLDs.

Cross-resistance

Cross-resistance between the older-generation fluoroquinolones is almost complete; limited evidence has suggested that the third-generation quinolones (notably moxifloxacin and gatifloxacin) may have enhanced clinical benefit – even in the presence of in vitro resistance to ofloxacin or ciprofloxacin – owing to their low MICs, enhanced antimycobacterial activity, and improved biochemical structure providing metabolic stability and long half-life, theoretically reducing the selection of resistant mutants (12, 13). This observation remains to be confirmed in controlled clinical trials.

Study findings on cross-resistance between the aminoglycosides and/or the polypeptides are contradictory. In summary, the genotypes associated with resistance to the aminoglycosides and the cyclic polypeptides are overlapping; therefore, a combination of an aminoglycoside and a polypeptide would be equivalent to using a single drug. However, a recent study (albeit small) showed that cross-resistance patterns and MICs vary among the different molecular mutations described (14). Although emerging evidence shows a clear association between drug resistance and specific molecular mutations, this association does not actually prove a role for the individual mutations in drug resistance. Generalizing resistance to a class of SLDs based solely on resistance to a single drug in the class may therefore be misleading, as summarized below (14).

- Isolates that acquire resistance to capreomycin are usually susceptible to kanamycin and amikacin. A small proportion may be resistant to kanamycin and

an even smaller proportion may be resistant to amikacin. The molecular basis for this observation has been described.

- Isolates that acquire resistance to amikacin essentially always have associated resistance to kanamycin and capreomycin. The molecular basis for this observation has been described.
- Isolates that acquire resistance to kanamycin show different levels of cross-resistance with amikacin and capreomycin. The molecular basis for some of these observations has been described;
- Isolates that acquire resistance to streptomycin are usually susceptible to kanamycin, amikacin and capreomycin. However, rare strains with apparently single-step mutations that confer resistance to both streptomycin and kanamycin have been observed, although the molecular mechanism is not known.

Table 2 summarizes current consensus on the reliability and reproducibility of DST for anti-TB drugs, based on a robust assessment of published studies combined with laboratory experience and expert opinion. The following broad criteria were used to assess the strength of available evidence, based on two or more criteria having been met for assigning a drug to a specific category.

- I. Extensive published studies, extensive multicentre laboratory review, broad intermethod agreement, high stability of drug powder in vitro, consistent DST reliability and reproducibility, extensive clinical outcome data.
- II. Extensive published studies, extensive multicentre laboratory review, limited intermethod agreement, variable DST reproducibility (and therefore reliability), variable stability of drug powder in vitro, less extensive clinical outcome data.
- III. Less extensive published studies, limited multicentre laboratory review, limited intermethod agreement, limited data on DST reproducibility and reliability, limited data on drug powder stability in vitro, limited clinical outcome data.
- IV. Limited or no published studies, limited multicentre laboratory review, limited data or questionable DST reproducibility (and therefore reliability), instability of drug powder in vitro, no clinical outcome data.
- V. No published studies, no multicentre laboratory review, reproducibility and reliability impossible to assess, unknown stability of drug powder in vitro, no clinical outcome data.

Table 2. Current status of DST methodology and critical concentrations for second-line DST

Drug group ^a	Drug	DST category	DST method available	DST critical concentrations (µg/ml)				
				Löwenstein-Jensen ^b	Middlebrook 7H10 ^b	Middlebrook 7H11 ^b	BACTEC460	MGIT960
Group 1 First-line oral anti-TB agents	Isoniazid	I	Solid, liquid	0.2	0.2	0.2	0.1	0.1
	Rifampicin	I	Solid, liquid	40.0	1.0	1.0	2.0	1.0
	Ethambutol	II	Solid, liquid	2.0	5.0	7.5	2.5	5.0
	Pyrazinamide	II	Liquid	-	-	-	100.0	100.0
Group 2 Injectable anti-TB agents	Streptomycin	II	Solid, liquid	4.0	2.0	2.0	2.0	1.0
	Kanamycin	II	Solid, liquid	30.0	5.0	6.0	4.0	-
	Amikacin	II	Liquid	-	-	-	1.0	1.0
	Capreomycin	II	Solid, liquid	40.0	10.0	10.0	1.25	2.5
	Viomycin	V	None	-	-	-	-	-
Group 3 Fluoroquinolones	Ciprofloxacin ^d	III	Solid, liquid	2.0	2.0	2.0	2.0	1.0
	Ofloxacin	III	Solid, liquid	2.0	2.0	2.0	2.0	2.0
	Levofloxacin	IV	Solid, liquid	-	2.0	-	-	2.0
	Moxifloxacin	IV	Liquid	-	-	-	0.5	0.25
	Gatifloxacin	IV	Solid	-	1.0	-	-	-
Group 4 ^c Oral bacteriostatic second-line anti-TB agents	Ethionamide	IV	Solid, liquid	40.0	5.0	10.0	2.5	5.0
	Prothionamide	IV	Solid, liquid	40.0	-	-	1.25	2.5
	Cycloserine	IV	Solid	40.0	-	-	-	-
	Terizidone	IV	None	-	-	-	-	-
	<i>P</i> -aminosalicylic acid	IV	Solid, liquid	1.0	2.0	8.0	2.0	-
	Thioacetazone	V	None	-	-	-	-	-
Group 5 ^c Antituberculosis agents with unclear efficacy (not recommended by WHO for routine use in MDR-TB patients)	Clofazimine	V	Liquid	-	-	-	4.0	-
	Amoxicillin/clavulanate	V	None	-	-	-	-	-
	Clarithromycin	V	None	-	-	-	-	-
	Linezolid	V	Liquid	-	-	-	1.0	1.0

^a WHO Guidelines for the programmatic management of drug-resistant tuberculosis (5).

^b Indirect proportion method recommended. Other solid media methods (resistance ratio, absolute concentration) have not been adequately validated for second-line drugs.

^c Routine DST for group 4 and 5 drugs is not recommended.

^d Ciprofloxacin is no longer recommended to treat drug-susceptible or drug-resistant TB (5).

^d Gatifloxacin only to be used in exceptional circumstances (5).

Rational use of DST in programmes for control of drug-resistant TB

The WHO *Guidelines for the programmatic management of drug-resistant tuberculosis* (5) call for rational strategies for case-finding and treatment of drug-resistant TB patients. Access to quality-assured DST is a prerequisite, with representative data from drug-resistance surveillance or surveys as a minimum to guide the design of treatment modalities.

The following considerations are relevant to the laboratory component of MDR-TB programmes.

Rapid rifampicin-resistance testing

In most settings, particularly where fixed-dose combination (FDC) first-line anti-TB drugs are used, resistance to rifampicin is almost invariably associated with resistance to isoniazid. Detection of rifampicin resistance therefore serves as a reliable (although not complete) proxy of MDR-TB. The advantages of rapid rifampicin testing include earlier identification of patients on inappropriate first-line regimens, prompt screening of patients at risk of MDR-TB, and early interruption of MDR-TB transmission.

Several tests for rapid detection of rifampicin resistance have been validated in laboratory-based studies and are currently being evaluated for feasibility, cost-effectiveness and cost-benefit in large-scale field demonstration studies. The use of line-probe assays for rapid detection of rifampicin resistance has recently been endorsed by a WHO expert group convened to evaluate the evidence base (WHO Expert Group on molecular line-probe assays for rapid screening of patients at risk of MDR-TB, communication, 31 March 2008).

The use of rapid rifampicin-resistance testing is recommended in high-risk MDR-TB settings (including high-burden HIV settings); however, confirmation of MDR-TB by conventional DST is still regarded as the gold standard, and adequate laboratory capacity to ensure a quality-assured diagnosis of MDR-TB remains a fundamental requirement. As no rapid tests for the diagnosis of XDR-TB are yet available, adequate laboratory capacity for second-line DST also remains a fundamental need.

Organization and funding of the laboratory network

TB laboratory networks conventionally have a pyramidal structure based on an appropriate number of peripheral (level I) laboratories capable of sputum-smear microscopy, an appropriate number of intermediate (level II) laboratories capable of mycobacteriological culture, and a single (or more than one in large countries) national (level III) laboratory capable of DST (15).

Although diagnostic and treatment programmes for drug-resistant TB are based on a range of strategies, *in vitro* DST plays a key role in all of these. As a minimum, laboratory capacity to reliably detect isoniazid and rifampicin resistance is a prerequisite. Routine DST for second-line drugs is **not** recommended unless the

required laboratory infrastructure and capacity have been established, rigorous quality assurance is in place, and sustainable proficiency has been demonstrated.

Proficiency in DST is a combination of laboratory technique and workload. In order to retain proficiency and expertise, it is recommended that second-line DST only be performed if at least 200 specimens from high-risk patients per year are expected. This implies centralization of laboratory services for second-line DST or outsourcing such services (e.g. to one of the laboratories in the SRL Network) where programmes involve small numbers of MDR-TB patients.

Adequate resource allocation (human and financial) to laboratory services is essential. For mycobacteriological culture and DST, one of the most neglected areas is currently the availability of sufficient, adequately qualified and trained laboratory staff. Funding should also be adequate to ensure a safe and functioning laboratory infrastructure with appropriate and well-maintained equipment and sufficient laboratory consumables.

Transport of infectious substances

Specimens from patients suspected of having MDR-TB as well as cultures of *M. tuberculosis* pose a significant public health risk if not properly transported. Cultures in particular constitute enriched infectious material containing large numbers of viable organisms, and the risk is compounded when cultures of resistant strains are transported.

International organizations such as the Universal Postal Union (UPU), the International Civil Aviation Organization (ICAO) and the International Air Transport Association (IATA) have developed strict guidelines and procedures to facilitate safe and expeditious shipment of infectious substances while at the same time ensuring public safety. These are guided by the United Nations *Model regulations on the transport of dangerous goods* (16). IATA issues *Infectious substances shipping guidelines* (17) regularly and imposes additional restrictions as necessary. These guidelines must be followed if a shipment is carried by members of IATA.

Exchange of *M. tuberculosis* cultures between countries (e.g. for diagnostic DST, retesting or proficiency testing) is always subject to international regulations, which include national import and export regulations specific to individual countries.

Infectious substances and diagnostic specimens likely to contain infectious substances require triple packaging in accordance with the United Nations *Model regulations* (16). Cultures of *M. tuberculosis* should be shipped in screw-capped tubes as primary watertight containers, enclosed in a second watertight container and finally enclosed in an appropriate outer shipping container. Petri-dish cultures and large volumes of liquid cultures must not be shipped.

Compliance with shipment requirements is the responsibility of the shipper, who must be familiar with national and international regulations.

Hand-carriage of infectious substances is strictly prohibited by international air carriers, as is the use of diplomatic pouches (17).

Surveillance and surveys using DST

Surveillance of anti-TB drug resistance is essential for providing information on the magnitude and trends in drug resistance, for developing appropriate treatment guidelines and for evaluating the impact of control programme interventions. In addition, survey or surveillance data on second-line drug resistance are recommended in designing appropriate treatment modalities.

Screening all MDR-TB strains for second-line drug resistance is recommended as a minimum (18) using the hierarchy of DST recommended below. Because of the reliability and reproducibility of DST for aminoglycosides, polypeptides and fluoroquinolones, and since resistance to these drugs defines XDR, DST of these second-line drugs constitutes a priority for surveillance and surveys, based on the history of specific drugs within the different categories used in a country. Second-line DST for all drugs in categories I and II (*Table 2*) is, however, very useful during surveillance and surveys to establish a baseline and inform treatment decisions, even if specific drugs in the different categories may not have been used in the past.

Hierarchy of DST under programmatic conditions

Programmes for management of drug-resistant TB should decide which drugs to test for resistance, based on the most appropriate strategy for designing treatment regimens and taking the above-mentioned constraints of SLD-DST into account. It is also recommended that DST be limited to those drugs used in individual country treatment strategies, i.e. there is little point in establishing DST capacity for second-line drugs that are unavailable or not recommended by country-specific programmes.

Box 1 provides an outline for systematic DST of first- and second-line anti-TB drugs under routine programmatic conditions.

Box 1 Systematic approach to implementation of DST under routine programmatic conditions

Step 1

Isoniazid
Rifampicin

Step 2

Ethambutol
Streptomycin
Pyrazinamide

Steps 1 and 2 may be merged if indicated by epidemiological considerations and/or treatment modalities (e.g. standardized or individualized MDR-TB regimens still involving first-line drugs) and if resources allow extended DST capacity.

Step 3

Amikacin, kanamycin, capreomycin
Ofloxacin (or fluoroquinolone of choice in treatment strategy)

Steps 1 and 3 may be merged in settings where XDR is a concern in order to enable the rapid identification of XDR-TB patients.

Given the variability in cross-resistance reported for the aminoglycosides, it is recommended that all aminoglycosides (including streptomycin) as well as capreomycin be tested for resistance where possible.

Selection of the most appropriate fluoroquinolone for use in treatment modalities should be based on a representative survey or surveillance data. Only one fluoroquinolone needs to be tested owing to extensive cross-resistance.

Routine DST for group 4 drugs (ethionamide, prothionamide, cycloserine, terizidone, *P*-aminosalicylic acid and for group 5 drugs (clofazimine, amoxicillin-clavulanate, clarithromycin, linezolid) is **not** recommended.

Biosafety and laboratory infection control

Transmission of TB – including drug-resistant TB – is a recognized occupational risk for laboratory workers. Adequate biosafety measures and prevention of laboratory-acquired infection are therefore paramount.

The relative hazards of infective microorganisms handled in the laboratory are classified by WHO according to the risk of causing human disease, the potential for laboratory spread and whether effective treatment and prevention measures are available. Related biosafety levels for laboratories have been defined, taking into account the pathogenic agent, the facilities available, and the equipment, practices and procedures required to ensure a safe laboratory working environment (19).

Irrespective of the risk, laboratory standards require that:

- appropriate and specific administrative controls (including good laboratory practice, standard operating procedures and accident management plans) are in place and enforced;
- appropriate engineering controls are being used and are functioning adequately as designed;
- personal protective equipment is appropriate for the tasks being performed;
- proper waste management procedures are in place;
- proper procedures for general laboratory safety (including physical, electrical and chemical safety) are in place and enforced.

M. tuberculosis is classified by WHO as a risk group 3 laboratory pathogen, causing serious human disease but with limited possibilities of (laboratory) spread and with effective treatment and preventive measures available (19). Mycobacteriological culture and DST procedures generate high concentrations of organisms that pose an increased risk of aerosol spread. Given the limited treatment options for MDR-TB (and even more so for XDR-TB), laboratory procedures for culture and DST therefore necessitate special containment through biosafety level 3 precautions.

Biosafety level 3 containment requires the strengthening of laboratory operations and safety programmes, specifically those related to laboratory design, use of specialized equipment to prevent or contain aerosols, and health surveillance of laboratory staff. Published guidelines on biosafety level 3 precautions should be rigorously followed (19) and expert engineering consultation sought when establishing laboratory infrastructure for second-line DST, taking the following essential aspects into consideration:

- Access to the containment laboratory must be restricted, preferably through an anteroom (i.e. a specific area designed to maintain the pressure differential between the laboratory and the adjacent space).
- A controlled ventilation system must be installed which maintains a directional airflow into the laboratory, supported by a visual monitoring device showing that proper directional airflow is maintained at all times.
- The building ventilation system must be constructed so that air from the containment laboratory is not recirculated to other areas within the building. This is usually achieved through high-efficiency particulate air (HEPA) filtration, which needs specialized engineering input, validation and maintenance to ensure compliance with safety standards.
- Essential equipment for mycobacteriological culture and DST includes appropriate and well-maintained biological safety cabinets, centrifuges and other safety equipment to meet biosafety level 3 precaution standards.

- Health and medical surveillance of laboratory personnel involved in mycobacteriological culture and DST is strongly recommended. Surveillance should include a detailed medical history, targeted baseline health assessment, monitoring of respiratory signs and symptoms, and a proactive plan for appropriate medical investigations when indicated.

Quality assurance

A diagnosis of MDR-TB or XDR-TB has profound implications for the individual patient. Accuracy of the laboratory diagnosis is therefore crucial and a comprehensive laboratory quality assurance programme must be in place to ensure the accuracy, reliability and reproducibility of DST results.

Central reference laboratories involved in programmes for drug-resistant TB should establish formal links with one of the laboratories in the Supranational Reference Laboratory Network (SRLN) to help ensure the quality of laboratory services and validation of DST results. The SRLN currently consists of 26 laboratories, including a global coordinating centre in Belgium.

The SRLN ensures DST standards by a system of external quality assurance that should preferably be established prior to the implementation of MDR-TB programmes. As a minimum, external quality assurance with an SRL should consist of the following:

- an initial assessment visit;
- proficiency testing with an adequate number of coded isolates;
- periodic rechecking of isolates obtained within the MDR-TB programme.

Proficiency testing by the SRLN involves regular distribution to national reference laboratories of panels of coded *M. tuberculosis* strains with predefined drug-resistance profiles. The test results of the reference laboratory are compared with the coded SRL results in blinded fashion and specific performance indicators (sensitivity, specificity, reproducibility) calculated for each drug and for the reference laboratory as a whole.

As a minimum performance indicator, proficiency testing should identify correctly resistance to isoniazid and rifampicin in more than 90% in two out of three recent rounds of panels.

The SRLN is in agreement that panels for second-line proficiency testing should not include XDR strains of *M. tuberculosis*. Rather, panels with different permutations of monoresistance to second-line drugs are currently being developed that will be compiled to allow reliable assessment of the overall capability of national reference laboratories to identify XDR-TB. Panels including isolates with second-line drug resistance will be made available through the SRLN in 2008.

Conclusions

Quality-assured laboratory services constitute the backbone of programmes for drug-resistant TB. Implementation of such programmes necessitate that governments and donors adequately fund appropriate and safe laboratory infrastructures in which well-trained staff working to clear standard operating procedures are able to deliver accurate and timely drug-resistance results. The need remains to improve DST for second-line drugs and to configure screening and diagnostic algorithms into rational management programmes for drug-resistant TB. In addition, accelerated expansion and integration of laboratory services as a core component of TB control programmes is required to achieve the aims of the global MDR-TB response and maximize the potential of new technological developments.

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