INT J TUBERC LUNG DIS 16(1):24–31

http://dx.doi.org/10.5588/ijtld.11.0223

The TDR Tuberculosis Strain Bank: a resource for basic science, tool development and diagnostic services

V. Vincent,*† L. Rigouts,‡ E. Nduwamahoro,‡ B. Holmes,§ J. Cunningham,* M. Guillerm,* C-M. Nathanson,* F. Moussy,* B. De Jong,‡ F. Portaels,‡ A. Ramsay*

***United Nations Children's Fund/United Nations Development Programme/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases (TDR), Geneva, Switzerland; †Institut Pasteur, Paris, France; ‡Institute of Tropical Medicine, Antwerp, Belgium; §National Collection of Type Cultures, London, UK**

SUMMARY

BACKGROUND: The Special Programme for Research and Training in Tropical Diseases recently launched a *Mycobacterium tuberculosis* strain bank (TDR-TB Strain Bank).

OBJECTIVE: To describe the TDR-TB Strain Bank, the characterisation of strains, bank management and the procedure for releasing materials.

RESULTS: The TDR-TB Strain Bank consists of 229 clinical *M. tuberculosis* isolates (single-colony derived cultures) plus five mycobacterial reference strains for purposes of identification. These are available as freeze-dried, viable strains or as heat-inactivated bacterial suspensions, quality controlled for purity, viability and authenticity. Isolates originated from diverse geographical settings and were selected for their resistance profiles against first- and second-line drugs. Low and high levels of resistance were determined by the minimum inhibitory concentrations of isoniazid, rifampicin, ethambutol, streptomycin, ofloxacin, kanamycin, capreomycin, ethionamide and para-aminosalicylic acid. Sequencing for drug resistance mutations was performed on the relevant sections of the *rpo*B, *kat*G, *inh*A, *emb*B, *rps*L, *rrs*, *gyr*A and *gyr*B genes. Typing using lineage-defining loci of mycobacterial interspersed repetitive unit–variable number tandem repeats indicated that the most important genetic lineages were represented.

CONCLUSIONS: The TDR-TB Strain Bank is a high quality bioresource for basic science, supporting the development of new diagnostics and drug-resistant detection tools and providing reference materials for laboratory quality management programmes.

KEY WORDS: strain bank; *Mycobacterium tuberculosis*; diagnostics development; drug susceptibility testing

TUBERCULOSIS (TB) remains a global public health emergency. Despite decades of effort, the pandemic is not under control and targets for reducing global incidence and mortality have not been met in all World Health Organization (WHO) regions.[1] Three major obstacles to TB control are recognised: the weak health systems that prevail in many of the disease-endemic countries, the high prevalence of human immunodeficiency virus (HIV) associated TB, and the increasing prevalence of multidrug-resistant TB.[1,2] Furthermore, extensively drug-resistant TB (XDR-TB) is being increasingly identified and poses a significant therapeutic challenge, with a high mortality rate. Since the first reported cases of XDR-TB in 2006 in South Africa,[3] XDR-TB has been recognised as a worldwide problem, with rates of as many as 10% of MDR-TB cases being recorded.[2,4]

New diagnostic tools are urgently needed to reliably identify TB cases—including HIV-associated TB cases, who tend to have lower bacillary loads—and detect critical forms of drug resistance at points of care in endemic countries. Robust laboratory quality management systems are, and will continue to be, needed to ensure continued optimal performance of diagnostic services. Beyond diagnostics, new classes of drugs are urgently needed to broaden our failing armamentarium of anti-tuberculosis drugs, and ultimately an effective vaccine is needed with improved efficacy relative to bacille Calmette-Guérin (BCG) vaccination.

Resistance mechanisms for most drugs are still poorly understood, except for rifampicin (RMP). Resistance to RMP is linked to a small 81 base pair sequence within a single gene; 96% of phenotypically resistant isolates contain mutations within this sequence.[5] The situation differs drastically for the other anti-tuberculosis drugs, where, in most cases, several

VV and LR are joint first authors. FP and AR are joint senior authors.

Correspondence to: Leen Rigouts, Mycobacteriology Unit, Institute of Tropical Medicine, Nationalestraat 155, 2000 Antwerpen, Belgium. Tel: (+32) 3 247 65 51. Fax: (+32) 3 247 63 33. e-mail: lrigouts@itg.be

Article submitted 4 April 2011. Final version accepted 15 July 2011.

genes are involved and mutations may be scattered over long gene sequences. These factors complicate the design of molecular tests to detect resistance against these drugs.

In a deliberate effort to support research on drug resistance mechanisms (that would underpin the development of new diagnostic and therapeutic tools) and the evaluation of new or existing tools, the Special Programme for Research and Training in Tropical Diseases (TDR), in collaboration with the Institute of Tropical Medicine (ITM), Antwerp, Belgium, and laboratories around the world, established a bank of *M. tuberculosis* isolates of known provenance. The main objectives of the project were to promote the development of new tools appropriate for use in disease-endemic countries, to facilitate the laboratory evaluation of new and existing diagnostics and drug resistance detection tests, and to provide reference materials in support of quality control and quality assessment programmes. The stock of the TDR Tuberculosis Strain Bank (TDR-TB Strain Bank) is available to end-users either as viable strains or as heat-inactivated bacterial suspensions.

The TDR-TB Strain Bank is part of a larger effort by TDR to provide clinical and microbiological reference materials to the global TB research community. The TDR-TB Specimen Bank, described previously,[6] complements the resources of the TDR-TB Strain Bank by providing researchers with specimens of sputum, serum, urine and saliva from well-characterised patients presenting to health care facilities across the globe with respiratory symptoms.

We describe here an audit of the TDR-TB Strain Bank and its establishment, the characterisation of strains, current stocks and the nature of the service it provides to the global TB research community.

MATERIALS AND METHODS

Strain selection

Nine laboratories contributed to the bank, which is hosted at the ITM. Strains were isolated from patients either diagnosed in their own countries or abroad, and were selected based on the diversity of their resistance patterns and geographic origin. The geographic origin of the 229 strains listed in Table 1 refers to the country where specimens were collected from patients. The country of origin is the only patient-related information included in the database.

The bank also includes the type strains of five *Mycobacterium* species, namely *M. tuberculosis* (H37Rv), *M. bovis, M. avium, M. fortuitum* and *M. terrae*, as positive and negative controls for tests identifying *M. tuberculosis*.

Characterisation

All strains selected for their inclusion in the TDR-TB Strain Bank were first sub-cultured on Dubos agar plates at the ITM. A single colony was picked from every culture, and subsequent propagations were kept to a minimum and performed on Löwenstein-Jensen (LJ) medium. All subsequent tests were carried out at the ITM from the single-colony derived cultures.

The phenotypic resistance pattern was analysed by establishing the minimum inhibitory concentration (MIC) of the following first-line drugs (FLDs): isoniazid (INH), RMP, ethambutol (EMB) and streptomycin (SM) on LJ using the following concentrations: 0.05, 0.2, 0.8, 1.6, 3.2 μg/ml for INH; 10, 20, 30, 40, 80, 120 μg/ml for RMP; 1, 2, 4, 8, 16 μg/ml for SM and 1, 2, 4, 8 μg/ml for EMB. The resistance patterns for the FLDs are indicated in Table 2 using the proportion method (PM), with the critical concentrations 0.2 μg/ml INH, 40 μg/ml RMP, 4 μg/ml SM and 2 μg/ml EMB. Second-line drugs (SLDs) included ofloxacin (OFL), kanamycin (KM), capreomycin (CPM), ethionamide (ETH) and p-aminosalicylic acid (PAS), so that one drug representative of the fluoroquinolone, aminoglycoside, polypeptide and thioamide classes of antibiotics was tested, according to WHO recommendations.[7] PAS was tested on LJ at 0.5 μg/ml. The other SLDs were tested on Middlebrook 7H11 agar medium at the following concentrations: OFL 2 μg/ml, KM 6 μg/ml, CPM 10 μg/ml,

Table 1 Geographical origin of 229 strains, by WHO region*

AFR (*n* = 48)		AMR (*n* = 49)		EMR (*n* = 7)		EUR (*n* = 33)		SEAR (*n* = 38)		WPR (*n* = 54)	
Country	*n*	Country	*n*	Country	*n*	Country	*n*	Country	*n*	Country	*n*
Burundi	8	Brazil	9	Morocco	5	Abkhazia	1	Bangladesh	34	China	1
Cameroon	1	Colombia	1	Pakistan	2	Azerbaijan	2	Nepal	4	Republic of South Korea	48
Central African Republic	1	Dominican Republic	1			Belgium	2			Philippines	4
Congo	9	Peru	38			Georgia	7			Thailand	1
Equatorial Guinea	1					Germany	14				
Guinea	1					Kazakhstan	3				
Niger	1					Portugal	1				
Nigeria	2					Spain	2				
Rwanda	18					Ukraine	1				
South Africa	6										

*The two XDR-TB strains (from Nigeria and India) are not included.
WHO = World Health Organization; AFR = WHO African Region; AMR = WHO Region of the Americas; EMR = WHO Eastern Mediterranean Region; EUR = WHO European Region; SEAR = WHO South-East Asia Region; WPR = WHO Western Pacific Region; XDR-TB = extensively drug-resistant tuberculosis.

Table 2 The 229 *M. tuberculosis* strains by type of resistance to the four FLDs: INH, RMP, EMB and SM

FLD resistance type	INH	RMP	EMB	SM	Total
1	S	S	S	S	59
2	R	S	S	S	10
3	S	R	S	S	13
4	S	S	R	S	5
5	S	S	S	R	17
6	R	R	S	S	19
7	R	S	R	S	10
8	R	S	S	R	18
9	S	R	R	S	2
10	S	R	S	R	5
11	S	S	R	R	2
12	R	R	R	S	17
13	R	R	S	R	15
14	R	S	R	R	8
15	S	R	R	R	0
16	R	R	R	R	29

FLD = first-line drugs; INH = isoniazid; RMP = rifampicin; EMB = ethambutol; SM = streptomycin; S = susceptible; R = resistant.

ETH 10 μg/ml. For all SLDs, resistance was determined using PM with a critical proportion of 1%. Two strains found to be XDR-TB, i.e., resistant to INH, RMP, KM or CPM, and OFL, were deliberately deleted from the catalogue of viable strains due to concerns about the risk of disseminating these virtually untreatable strains. However, these strains were not destroyed and the DNA is available for further molecular studies.

Eleven strains susceptible to most FLDs, but with various SLD resistance patterns, were added to enrich the bank with SLD-resistant strains while avoiding the inclusion of XDR-TB strains. Strains found resistant to any SLD using PM were further investigated by determination of the MIC using the following concentrations on LJ medium: 0.05, 1, 2, 4 and 8 μg/ml for OFL; 0.025, 0.05, 1, 2 and 4 μg/ml for gatifloxacin (GFX) and moxifloxacin (MFX); 7.5, 15, 30, 60 and 120 μg/ml for KM; and 10, 20, 40, 80 and 160 μg/ml for CPM and amikacin (AMK).

The genetic characterisation of the strains included sequencing of genes involved in resistance to FLDs: *rpo*B (codon 209–694), *kat*G (codon 268–328), *inh*A (position −148 to +60), *emb*B (codon 254–413), *rps*L (codon 9–96) and *rrs* (position 68–990). For strains found resistant to an SLD, the genes *gyr*A (codon 1–137), *gyr*B (codon 478–714) and *rrs* (position 1028–537) were sequenced in addition.

Strains were typed using selected loci of the MIRU-VNTR (mycobacterial interspersed repetitive units–variable number tandem repeat) loci according to Supply et al. to determine their genetic diversity and representativeness of genetic families.[8,9] Moreover, spoligotyping was performed for strains that showed a Beijing-like MIRU pattern to ascertain strains belonging to the Beijing family. The manual spoligotyping method was applied as described by Kamerbeek et al.[10]

Strain preservation

As the bank is intended to be a long-term resource, storage of the strains in a way that guarantees viability and minimises genetic drift are critical. It was therefore decided that all strains would be lyophilised in two different types of lots (Figure 1):

1 Mother lots to secure the strains for long-term storage with 20 sealed glass ampoules per strain. This preparation was first entrusted to the National Collection of Type Cultures (NCTC) in London, until ITM became properly equipped for safe freeze-drying of Risk Group 3 pathogens in its biosafety level 3 laboratory. Mother lots are stored in two distinct locations at ITM and will only be used when distribution lots of a strain are depleted.
2 Distribution lots, i.e., the material to be distributed to end-users, are prepared from one ampoule of the mother lot of each strain, and consist of 20–50 glass and/or rubber-capped vials per strain.

To anticipate client requests for strain panels for specific quality assurance (QA) objectives, such as internal quality controls (IQC) or external quality assurance (EQA) for phenotypic or genotypic tests, a few selected strains were prepared in larger stocks. For these strains, up to 50 mother-lot ampoules and at least 30 distribution-lot entities were prepared. The distribution lots of the selected strains consist of rubber capped glass vials (instead of sealed ampoules) for easier handling by end-users.

The system allows the TDR-TB Strain Bank to guarantee the authenticity of strains over decades. Lots are prepared from strain cultures grown on LJ,

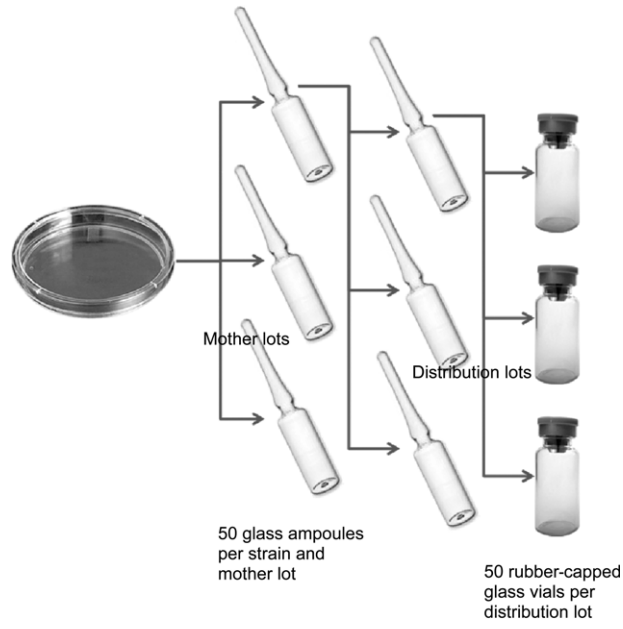

Figure 1 The production of mother and distribution lots allows the bank to keep the integrity of the strains for an estimated 100 years.

either from the sub-culture of a single colony and limited to two to three passages for the mother lots, or from a mother lot for the distribution lot. For ampoules/vials produced at ITM, scraped colonies were suspended in a sterile solution of saccharose 10%, neopeptone 5% and fetal bovine serum (vol 1/1/1) and submitted to freeze-drying. Ampoules and vials were sealed under vacuum conditions. Freeze-dried lots were stored in the dark at room temperature.

Preparation of heat-inactivated bacterial suspensions

Cells grown on LJ from a distribution lot were scraped and resuspended in 1× Tris-ethylenediaminetetraacetic acid (EDTA) buffer (10 mM Tris, 1 mM EDTA, pH 7.5). From suspensions of 1 mg/ml, 0.5 ml volumes were prepared in O-ring capped tubes, which were then heat-inactivated at 100°C for 15 min and stored at −18°C. Ten tubes were prepared for each strain.

Quality control of mother and distribution lots

One vial of each mother lot was quality controlled for purity, viability and authenticity. For purity, freeze-dried cells were resuspended in sterile distilled water and inoculated onto blood agar plates to detect growth of bacteria other than tubercle bacilli. For viability, resuspended bacilli were inoculated onto LJ in serial 10-fold dilutions and incubated at 37°C. Colonies were counted to evaluate the viability of the bacilli. For authenticity, cells scraped from one LJ tube of the viability test were resuspended in 1× TE buffer and heat-inactivated. Authenticity tests targeted an unusual number of repetitions at an MIRU locus, a rare spoligotyping pattern or an infrequent mutation in a gene conferring resistance to an antibiotic.

Mother lots were released when these three tests used to assess their quality provided satisfactory results, failing which the mother lots were prepared again from the original strain stored at −70°C in Dubos broth medium containing Middlebrook albumin-dextrose-catalase enrichment and 10% glycerol, which is maintained as a back-up system. Quality control of the distribution lots was performed using the same three tests described above on one vial of each distribution lot. Similarly, distribution lots were released only when quality controls provided satisfactory results.

Quality control of heat-inactivated bacterial suspensions

The heat-inactivated bacterial suspensions were checked for DNA integrity by electrophoresis on agarose gel. DNA authenticity was checked as described above.

Maintenance of bank inventory

All scientific information regarding the strains in the bank is stored in an appropriate, protected database. Raw data on strain characteristics, quality control data, as well as data on stock of ampoules and vials are also maintained in standard forms and a logbook.

RESULTS

The TDR-TB Strain Bank comprises a total of 229 *M. tuberculosis* strains selected for their resistance profile against FLDs and SLDs and their genetic diversity, plus five mycobacterial reference strains for identification purposes.

Geographic distribution and genetic diversity

Five of the six WHO regions are equally represented, comprising 33–54 strains each (Table 1). The East Mediterranean Region, however, is underrepresented, with only seven strains included. Within each region, two to 10 countries were included. Bangladesh, Peru and the Republic of South Korea together represent 51% of the collection, with respectively 34, 38 and 48 strains. In terms of genetic diversity assessed by MIRU-VNTR typing, the most important lineages are present (additional data provided on request).

FLD resistance

The bank contains 80 (34.9%) MDR-TB strains and 59 (25.8%) strains susceptible to the four FLDs tested (Table 3). It was intended to collect strains with all 16 possible combinations of susceptibility and resistance to the FLDs (Table 2). Apart from type 15 FLD resistance (resistant to RMP+EMB+SM), all resistance combinations were represented in the remaining 15 types, with 2–59 strains per type (mean 14 strains per type). Polyresistant, non-INH-resistant strains (types 9, 10, 11) are less frequent than polyresistant INH-resistant strains (types 7, 8 and 9).

The bank has few isolates with low-level RMP resistance (Table 3). Among the 10 strains with an MIC of 80 μg/ml, one strain was a wild-type (WT) *rpo*B gene, whereas four had a Ser531Leu mutation, one a Leu533Pro, one a Leu511Pro, one an Ile572Phe and two an Asp516Tyr mutation. The latter three mutations were not seen among our strains with MICs >80 μg/ml. It has recently been demonstrated that low-level RMP-resistant strains yield highly discordant results, depending on the culture medium used, and that the LJ medium yielded the most reproducible results.[11] Interestingly, the initial propagation of strains in the TDR-TB Strain Bank was performed on LJ.

Among the 126 INH-resistant strains, 19 showed low-level resistance (MIC 0.8 μg/ml; Table 3). Four of these had WT sequences for both *kat*G and *inh*A, 12 (63%) had a C→T mutation at the *inh*A upstream position −15, one a G→A mutation at position −9, and two a Ser315Asn mutation in *kat*G. None of these mutations were seen among the 46 strains with MIC of ⩽0.2 μg/ml. On the other hand, C-15T was observed in only seven high-level resistant strains

Table 3 Phenotypic and genotypic traits of 218 *M. tuberculosis* strains selected for their first-line drug resistance profile

	Any phenotypic resistance			Low-level resistance only		High-level resistance only	
Drug	Strains *n*	Strains with known resistance-conferring mutations *n* (%)	Strains showing mutations with unclear relevance *n* (%)*	MIC and number of strains µg/ml (*n*)	Strains with known resistance-conferring mutations %†	MIC and number of strains µg/ml (*n*)	Strains with known resistance-conferring mutations %†
INH	126	117 (92.9)‡ 116 (92.1)†	1 (0.8)‡ 1 (0.8)†	0.8 (19)	*inh*A = 68 *kat*G = 10.5	1.6 (7) ≥3.2 (100)	*inh*A = 42.8 *kat*G = 42.8 *inh*A = 4 *kat*G = 93
RMP	102	101 (99)‡ 96 (94.1)†	0‡ 5 (4.9)†	80 (10)	*rpo*B = 90	120 (5) >120 (87)	*rpo*B = 100 *rpo*B = 100
SM	92	45 (48.9)‡ 44 (47.8)†	2 (2.2)‡ 3 (3.3)†	8 (32)	*rrs* = 6.25 *rps*L = 0	16 (13) >16 (47)	*rrs* = 23.1 *rps*L = 0 *rrs* = 19.1 *rps*L = 63.8
EMB	72	47 (65.3)‡ 43 (59.7)†	11 (15.3)‡ 15 (19.4)†	4 (33)	*emb*B = 35.3	8 (24) >8 (15)	*emb*B = 75 *emb*B = 86.7

*In the absence of a known mutation.
†Based on the list of high-confidence mutations registered with the TBDReaMDB database (update April 12, 2010) available at http://www.tbdreamdb.com/.
‡Based on the list of all mutations registered with the TBDReaMDB database available at http://www.tbdreamdb.com/ (updated April 12, 2010).
MIC = minimum inhibitory concentration; INH = isoniazid; RMP = rifampicin; SM = streptomycin; EMB = ethambutol; MIC = minimal inhibitory concentration.

without concurrent *kat*G mutation. The proportion of *kat*G mutations correlates with increases in MIC.

Among the 92 SM-resistant isolates with MIC tested, only half show a mutation in the *rrs* or *rps*L genes (Table 3), with high-level resistance (MIC >16 µg/ml) associated with mutations in the *rps*L gene (63.8%). Association of mutations in the 530 loop or 900 region of the *rrs* gene with phenotypic resistance to SM is not as straightforward: 6.25% of low-level and 19.1–23.1% of high-level resistant strains carry an *rrs* mutation. Furthermore, the C491T mutation in the *rrs,* which is quoted as high confidence in the TBDReaMDB database (www.tbdreamdb.com), was seen in only one low-level resistance strain, but also in six susceptible strains, of which five had MIC ≤1 µg/ml (data not shown).

Although few strains with phenotypic resistance to RMP, INH or SM showed mutations with unclear relevance (0.8–4.9%), this number was higher among phenotypic EMB-resistant strains (19.4%). Furthermore, the so-called high-confidence mutations, such as Met306Val, appear not to be fully specific for phenotypic drug resistance, as they were also encountered in non-resistant strains (16.7% of strains showing an MIC of 2 µg/ml and 2.9% of strains with an MIC ≤1 µg/ml; data not shown).

Apart from the known and probable resistance-conferring mutations, an important proportion of strains showed single nucleotide polymorphisms (SNPs), particularly in the *rpo*B and *emb*B genes (data not shown). These mutations occurred alone or in combination with high-confidence mutations, and in both phenotypically susceptible and resistant strains.

SLD resistance

Among the 80 MDR-TB strains, 13 were resistant to one of the four SLDs tested, and five to more than one SLD, but were not XDR-TB (Table 4). All seven ETH-resistant strains carried a C-15T mutation in the promoter region of the *inh*A gene. In three of these

Table 4 Second-line drug resistance profiles for 80 MDR-TB strains

FLD type	Total	Resistant to 1 SLD	Resistant to >1 SLD	Susceptible to all SLDs tested	SLD testing failed
6	19	2 ETH-resistant	0	15	2
12	17	2 ETH-resistant 1 OFX-resistant	1 CPM+KM-resistant 1 ETH+PAS+CPM-resistant	12	0
13	15	1 ETH-resistant 1 KM-resistant 1 PAS-resistant	1 ETH+PAS-resistant	11	0
16	29	2 OFX-resistant 1 CPM-resistant 2 PAS-resistant	1 OFX+PAS-resistant 1 CPM+KM-resistant	22	0
Total	80	13	5	60	2

MDR-TB = multidrug-resistant tuberculosis; FLD = first-line drug; SLD = second-line drug; CPM = capreomycin; ETH = ethionamide; KM = kanamycin; OFX = ofloxacin; PAS = para-aminosalicylic acid.

cases, ETH resistance was associated with low-level INH resistance, whereas the remaining three showed high-level INH resistance; the strain with an INH MIC of 1.6 μg/ml had no additional *kat*G mutation, whereas the two strains with an INH MIC of 3.2 μg/ml carried a Ser315Thr mutation of the *kat*G gene in addition. ETH resistance was associated with resistance to PAS or PAS+CPM in two cases.

Among the 11 non-MDR-TB strains with resistance to SLDs, while being susceptible to most FLDs, seven showed phenotypic resistance to OFL: one with low-level resistance (MIC 4 μg/ml) without mutation in the *gyr*A and *gyr*B genes, and six with high-level resistance (>8 μg/ml). Two of the latter had no mutations, two showed a high-confidence mutation, and two showed genotypic heteroresistance with a mixture of WT and mutant sequence of the *gyr*A gene. MIRU-VNTR typing did not reveal the presence of multiple *M. tuberculosis* strains.

Among the six KM-resistant strains, phenotypic resistance to KM was associated with CPM and AMK resistance in four of six strains, all yielding an A1401G mutation in the *rrs* gene and high-level resistance. Of the two remaining strains, one showed low-level KM and CPM resistance without mutations in the *rrs* gene, whereas the other was highly resistant to KM, but not to AMK and CPM, despite the presence of an A1401G mutation. The above findings highlight the need for further research for a better understanding of the SLD resistance mechanism and the role of possible additional genes that contribute to phenotypic resistance.

DISCUSSION

Bank content/quality of the bank

The TDR-TB Strain Bank constitutes a unique resource of *M. tuberculosis* strains, comprising 229 strains extensively studied in relation to their drug susceptibility testing (DST) patterns against FLDs and SLDs, with determination of low and high levels of resistance based on MICs and of associated mutations in the genes involved in drug resistance mechanisms. The bank includes isolates from all over the world, as strains were originally isolated from patients diagnosed in all WHO regions, totalling 31 countries. The materials of the bank, both viable strains and heat-inactivated bacterial suspensions, are available to end-users. To our knowledge, other biobanks, national or large culture collections open to the public, maintain only a limited number of *M. tuberculosis* strains and do not document drug patterns or gene sequences. As the main objectives of the strain bank are to support the development of novel technologies for DST, evaluation of DST methods, and the implementation and management of QA of phenotypic and molecular DST methods, we deliberately focused on the purity of isolates and advanced molecular characterisation. Strains were re-isolated and sub-cultured from a single colony, and do not necessarily reflect the original *M. tuberculosis* populations present in patient lesions.

Heat-inactivated bacterial suspensions can be used as DNA sources. Although DNA was not purified, this raw material is adequate for standard DNA amplification and evaluation or EQA of molecular tools designed for the diagnosis of TB resistance. It facilitates access to materials for end-users who do not have a biosafety level 3 laboratory in which to handle viable *M. tuberculosis* strains. Heat-inactivated bacterial suspensions are of special interest, as they facilitate inclusion of the two hazardous XDR-TB strains in the bank.

The development of the bank followed the best practices for repositories.[12] Specific quality controls were implemented at every stage of preparation of the materials. The viability, purity and authenticity of each mother and distribution batches, as well as DNA integrity and authenticity of heat-inactivated cell suspensions, were carefully checked. The bank is located and maintained at the Mycobacteriology Unit of ITM, one of the laboratories with the best expertise in the field. The ITM laboratory currently coordinates the EQA of the WHO/International Union Against Tuberculosis and Lung Disease (The Union) global project on anti-tuberculosis drug resistance surveillance launched in 1994.[13] As the production of freeze-dried mother and distribution lots minimises the risk of genetic variations from one request to the other, users are assured of long-term access to materials with the same characteristics over time, especially from one request to the other. In this regard, the TDR-TB Strain Bank is much more than a collection of isolates in a reference TB laboratory, which indeed may have much larger sets of *M. tuberculosis* isolates.

Bank management

The managerial organisation of the TDR-TB Strain Bank profited from the experience of managing the TDR-TB Specimen Bank.[6] TDR manages the bank with the advice of a review committee composed of TDR staff and a panel of external specialists with expertise in basic science on drug resistance and evolution of *M. tuberculosis*, evaluation of diagnostic tests and public health TB laboratories. As the bank is not a static resource and should develop over time, the committee is responsible for reviewing the composition of the strain bank, making recommendations regarding the need for additional strains, and providing technical support regarding bank procedures, especially needs for extended characterisation of strains. The review committee also advises TDR on how to make the strain bank more responsive to user needs and on advocacy and communication strategies.

The committee is also responsible for reviewing all projects submitted to TDR requesting the use of the strain bank resources. The committee approves, rejects

or defers requests based on their relevance and scientific quality, as described in the Material Request Form below. Criteria for approval are similar to those used for the TDR-TB Specimen Bank:[6] preliminary data supporting the project, technology deemed to be suitable for resource-limited settings, and information about tests on which the strains will be used. All information provided by the applicant to the WHO in or in connection with the Material Request Form is treated by TDR and the Review Committee members as strictly confidential and proprietary of the applicant. For an overview of the whole process, see Figure 2.

Material Request Form

A specific material request form has been developed to submit requests to TDR (e-mail InfoSpecimen@who.int). On this form, the applicant describes the project for which isolates are being requested (e.g., research, diagnostic development, diagnostic evaluation, quality management of DST methods), with focus on expected outcomes and impact of the work on diagnosis of resistance. Specific attention is accorded to laboratory biosafety laboratory level and practice. For requests for freeze-dried vials (i.e., 50–200 μl of about 10^8 viable *M. tuberculosis* bacilli), the applicant must provide TDR with recent evidence (<12 months) of a satisfactory inspection of the facility for the safe handling, manipulation, use, storage and disposal of *M. tuberculosis* strains from the relevant national or international authority or accredited certifying agent.

The applicant's acceptance of responsibility includes, among other liabilities, ensuring that only qualified personnel will work with the materials in proper facilities and will not dispose of the materials to any third party.

The Material Transfer Agreement to be signed by the recipient stipulates that the material will be used exclusively for the purpose of the project approved by TDR, that the material and related information be treated as confidential and proprietary to TDR, will not be used commercially and will not be made available to any third party. The recipient is free to file patent applications and publish the results of their work, acknowledging TDR as appropriate.

Release of the materials

After approval, requests are sent to ITM for release and shipping of the strains or heat-inactivated bacterial suspensions. The materials that best fit the characteristics requested by the applicant are selected by TDR and ITM. The current release fees (in 2011) are US$120 per item for profit organisations and US$60 for non-profit organisations.

TDR will be the primary site for communication among end-users and ITM. All publications and presentations of the results using the materials will contain an acknowledgement of the TDR-TB Strain Bank.

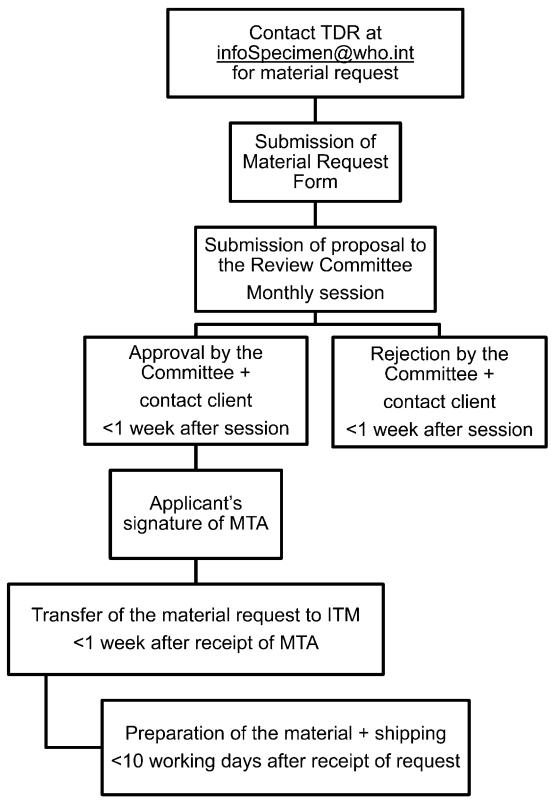

Figure 2 Process for request for approval of and release of tuberculosis strains. TDR = World Health Organization Special Programme for Research and Training in Tropical Diseases; MTA = material transfer agreement; ITM = Institute of Tropical Medicine.

CONCLUSION

To date, the TDR-TB Strain Bank has already been used for two major projects: the development of a new technology for the rapid and sensitive detection of tubercle bacilli and RMP resistance, the Xpert® MTB/RIF assay (Cepheid, Sunnyvale, CA, USA),[14,15] and the implementation of EQA of line-probe assays within the framework of the TB Pan Net Project, the European network for study and clinical management of TB drug resistance (http://www.tbpannet.org/). With regard to EQA issues, the TDR-TB Strain Bank can be used to extend the EQA distributions organised by the supranational reference laboratory (SRL) network for the WHO/The Union global project on anti-tuberculosis drug resistance surveillance to laboratories not linked to the SRL network, especially private laboratories and non-governmental organisations, and give them access to reference materials for phenotypic or molecular DST.

We expect to enrich the database with additional information, such as additional gene sequences that will result from the investigations carried out by users. TDR will be pro-active in seeking adequate feedback from applicants. The additional information

will be recorded in the database, with specific attributions to authors. This interaction with developers of faster and simpler methods of DST will benefit the TDR-TB Strain Bank, and its future end-users, with the availability of a bioresource of high quality.

Acknowledgements

G-H Bai, D Cirillo, S Dorman, F Drobniewski, S Gagneux, S Hoffner, M Iseman, K M Kam, J McFadden, A Laszlo, M Perkins, P del Portillo, F Portaels, T Shinnick, P van Helden and V Vincent were members of the successive Review Committees during the years of development of the TDR-TB Strain Bank project; their contribution is gratefully acknowledged. M T Mendoza (University of Philippines, Manila, Philippines); E Gotuzzo (Instituto de Medicina Tropical 'Alexander von Humboldt', Lima, Peru); G-H Bai, (Korean Institute of Tuberculosis, Seoul, Republic of Korea); N M Casabona (Clínica Hospital Universitari 'Vall d'Hebron', Barcelona, Spain); K Feldmann (Kuratorium Tuberkulose in der Welt, Gauting, Germany); A Sloutsky (Massachusetts State TB Laboratory Institute, Boston, MA, USA); M A da Silva Telles (Instituto Adolfo Lutz, Sao Paulo, SP, Brazil); and K Weyer (Medical Research Council, Pretoria, South Africa) are gratefully acknowledged for providing strains. S Brimble, A Deheer-Graham and P Olokesusi at NCTC are gratefully acknowledged for their efforts in freeze-drying the mother lots. The TDR-TB Strain Bank has been funded in part by the Bill & Melinda Gates Foundation and the United States Agency for International Development through grants awarded to the United Nations Children's Fund/United Nations Development Programme/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (grant nos. 3636/9900727 and AAGG-00-99-00005-31, respectively). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

1 World Health Organization. Global tuberculosis control: epidemiology, strategy, financing: WHO report 2009. WHO/HTM/TB/2009.411. Geneva, Switzerland: WHO, 2009.
2 World Health Organization. Anti-tuberculous drug resistance in the world. Report no. 4. The WHO/IUATLD global project on anti-tuberculosis drug resistance surveillance. WHO/HTM/TB/2008.394. Geneva, Switzerland: WHO, 2008.
3 Singh J A, Upshur R, Padayatchi N. XDR-TB in South Africa: no time for denial or complacency. PLoS Med 2007; 4: e50.
4 Shah N S, Wright A, Bai G-H, et al. Worldwide emergence of extensively drug-resistant tuberculosis. Emerg Infect Dis 2007; 13: 380–387.
5 Telenti A, Imboden P, Marchesi F, et al. Detection of rifampicin-resistance mutations in *Mycobacterium tuberculosis*. Lancet 1993; 341: 647–650.
6 Nathanson C-M, Cuevas L E, Cunningham J, et al. The TDR Tuberculosis Specimen Bank: a resource for diagnostic test developers. Int J Tuberc Lung Dis 2010; 14: 1461–1467.
7 World Health Organization. Policy guidance on drug-susceptibility testing (DST) of second-line antituberculosis drugs. WHO/HTM/TB/2008.392. Geneva, Switzerland: WHO, 2008.
8 Supply P, Allix C, Lesjean S, et al. Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of *Mycobacterium tuberculosis*. J Clin Microbiol 2006; 44: 4498–4510.
9 Supply P, Mazars E, Lesjean S, Vincent V, Gicquel B, Locht C. Variable human minisatellite-like regions in the *Mycobacterium tuberculosis* genome. Mol Microbiol 2000; 36: 762–771.
10 Kamerbeek J, Schouls L, Kolk A, et al. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. J Clin Microbiol 1997; 35: 907–914.
11 Van Deun A, Barrera L, Bastian I, et al. *Mycobacterium tuberculosis* strains with highly discordant rifampin susceptibility test results. J Clin Microbiol 2009; 47: 3501–3506.
12 International Society for Biological and Environmental Repositories. 2008 best practices for repositories. Collection, storage, retrieval and distribution of biological materials for research. Bethesda, MD, USA: ISBER, 2008.
13 World Health Organization. Anti-tuberculosis drug resistance in the world. The WHO/IUATLD global project on anti-tuberculosis drug resistance surveillance. WHO/TB/97.229. Geneva, Switzerland: WHO, 1997.
14 Blakemore R, Story E, Helb D, et al. Evaluation of the analytical performance of the Xpert MTB/RIF assay. J Clin Microbiol 2010; 48: 2495–2501.
15 Helb D, Jones M, Story E, et al. Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol 2010; 48: 229–237.

RÉSUMÉ

CONTEXTE : Lancement de la banque de souches de *Mycobacterium tuberculosis* (banque de souches TDR-TB) par le Programme spécial de Recherche et de Développement concernant les Maladies tropicales.

OBJECTIF : Décrire la banque de souches TDR-TB, la caractérisation des souches, la gestion de la banque et la procédure d'obtention du matériel biologique.

RÉSULTATS : La banque de souches TDR-TB consiste en 229 isolements cliniques de *M. tuberculosis* (cultures dérivées du repiquage d'une colonie) plus cinq souches de référence d'autres espèces mycobactériennes. Le matériel est disponible soit sous forme de souches viables lyophilisées, soit sous forme de suspensions bactériennes inactivées par la chaleur. Les contrôles de qualité garantissent la pureté, la viabilité et l'authenticité du matériel. Les isolements proviennent de diverses origines géographiques et ont été sélectionnés pour leurs profils de résistance aux médicaments de première et de seconde ligne. Les niveaux de résistance, basse ou élevée, ont été déterminés par la méthode des concentrations minimales inhibitrices pour l'isoniazide, la rifampicine, l'éthambutol, la streptomycine, l'ofloxacine, la kanamycine, la capréomycine, l'éthionamide et l'acide para-aminosalicylique. Le séquençage pour recherche de mutations conférant la résistance aux médicaments a été effectué sur des fragments des gènes *rpo*B, *kat*G, *inh*A, *emb*B, *rps*L, *rrs*, *gyr*A et *gyr*B. Le typage utilisant les loci de définition des lignées génétiques des unités mycobactériennes intercalées répétées–nombre variable de répétitions en tandem montre que les lignées les plus prévalentes sont représentées.

CONCLUSION : La banque de souches TDR-TB est une ressource biologique de qualité pour la recherche fondamentale, le développement de nouveaux outils diagnostiques et de détection des résistances aux antibiotiques et la mise à disposition de matériel de référence pour les programmes d'assurance qualité des laboratoires.

RESUMEN

MARCO DE REFERENCIA: En el Programa especial de Investigación y Capacitación sobre Enfermedades tropicales (TDR-TB) se creó un banco de cepas de *Mycobacterium tuberculosis.*

OBJETIVO: Describir el banco de cepas de *M. tuberculosis* del TDR, la caracterización de las cepas, la gestión del banco y los procedimientos de difusión de los materiales.

RESULTADOS: El banco de cepas del TDR contiene 229 aislados clínicos de *M. tuberculosis* (cultivos derivados de una sola colonia) y cinco cepas de micobacterias de referencia que se utilizan en la identificación. Todas las cepas se encuentran ya sea en forma liofilizada, como cepas viables o como suspensiones micobacterianas inactivadas por calor, cuya calidad se controla en materia de pureza, viabilidad y autenticidad. Los aislados provienen de entornos geográficos diversos y se escogieron en función de su perfil de resistencia a los medicamentos antituberculosos de primera y segunda línea. Los grados de resistencia alta o baja se establecieron mediante las concentraciones inhibitorias mínimas de isoniazida, rifampicina, etambutol, estreptomicina, ofloxacino, kanamicina, capreomicina, etionamida y para-aminosalicilato. Se llevó a cabo una secuenciación en busca de las mutaciones que codifican la farmacorresistencia en las regiones de interés de los genes *rpo*B, *kat*G, *inh*A, *emb*B, *rps*L, *rrs*, *gyr*A y *gyr*B. La genotipificación con marcadores de las unidades micobacterianas repetidas dispersas y las secuencias repetidas en serie de número variable indicó que en la colección están representados los principales linajes genéticos.

CONCLUSIÓN: El banco de cepas de micobacterias del TDR constituye un recurso biológico de gran calidad para la investigación fundamental, respalda el desarrollo de nuevos instrumentos diagnósticos y de detección de farmacorresistencia y provee además materiales de referencia a los programas de gestión del control de la calidad de los laboratorios.