

METHODS

CHAPTER 2

ANTI-TUBERCULOSIS DRUG RESISTANCE IN THE WORLD

2 METHODS

Methods used in the Global Project have been extensively described. For this report, therefore, the methods are only summarized, while changes and new developments are described in detail. For more detailed information, the reader is encouraged to consult the following publications: *Anti-tuberculosis Drug Resistance in the World: the WHO/IUATLD Global Project on Anti-tuberculosis Drug Resistance Surveillance (WHO/TB/97.229)*,⁴¹ *the New England Journal of Medicine 1998; 338:1641–1649*,² *the WHO Guidelines for Surveillance of Drug Resistance in Tuberculosis (WHO/TB/96.216)*,²⁸ *the International Journal of Tuberculosis and Lung Disease 1998; 2:71–89*,²⁹ and *the International Journal of Tuberculosis and Lung Disease 1997; 1:231–238*.⁴⁷

2.1 BACKGROUND OF THE GLOBAL PROJECT

WHO and the IUATLD developed a set of standardized methods of surveillance in 1994. They also established an international Working Group in the same year. The Working Group delineated a system to ascertain the global magnitude of the problem of anti-tuberculosis drug resistance. This system comprised two components: 1) standardized surveys/surveillance implemented on representative samples of TB patients at country or region-within-country level, i.e., state-wide, province-wide; and 2) proper bacteriological methodology in national laboratories through an international system of proficiency testing. Guidelines for the performance of anti-tuberculosis drug resistance surveillance were developed.^{28,29} These guidelines introduced standard definitions and the procedures to implement drug resistance surveillance. They are currently available in Chinese, English, French, Italian, Russian, and Spanish.

2.2 UPDATE ON THE SUPRANATIONAL REFERENCE LABORATORY (SRL) NETWORK

The WHO/IUATLD supranational reference laboratory (SRL) network was created in 1994, to ascertain the accuracy of the susceptibility test methods used in different laboratories across the world, and to allow comparability of the surveillance data gathered in countries participating in the Global Project. Today, the network has evolved and 23 SRLs actively participate. While in 1994–1998 the Canadian Laboratory Centre for Disease Control (LCDC) acted as the coordinating centre, a new network coordinating centre was appointed in 1999, namely The Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium. Two new SRLs have been incorporated while two others are no longer part of the network. Other laboratories are in the process of evaluation to become SRLs. As of September 1999, the network has SRLs located in the Americas (Argentina, Chile, and United States), Europe

(Belgium, Czech Republic, France, Germany, Italy, The Netherlands, Portugal, Spain, Sweden, United Kingdom), Africa (Algeria, South Africa), Asia (India, Japan, and the Republic of Korea), and Oceania (Australia) (see Table 1).

Due to the success of the SRL network, requests from many laboratories around the globe to participate in the network became increasingly difficult to handle. As a result, WHO and IUATLD stimulated the creation of regional sub-networks of laboratories within this global network. For the purpose of proficiency testing one or various SRLs located in specific geographic regions coordinate the distribution of the strains received from the global coordinating centre (Belgium SRL) to other laboratories not directly linked with the global coordinating centre. As of 1999, sub-networks exist or are underway in Africa (coordinated by the SRL in South Africa), Asia (coordinated by the SRL in the Republic of Korea), Europe (coordinated by the SRLs in France, Germany, Sweden, and United Kingdom), and Oceania (coordinated by the SRL in Australia). This umbrella system has brought into the network more than 100 laboratories worldwide.

Inter-laboratory testing of the proficiency of drug susceptibility testing (DST) is conducted regularly on an annual basis within the network. Six rounds of strain exchange have been carried out between 1994 and 1999. Up to 1998, in each round, the then coordinating centre in Canada sent two identical sets of ten clinical isolates of *M. tuberculosis* (20 cultures) to all SRLs. In 1999 this exercise was conducted for the first time by the newly appointed coordinating centre (Belgium SRL). The SRLs are asked to test the susceptibility pattern of the reference strains with their usual methodology, and classify the cultures as *resistant or susceptible*. The susceptibility results of *M. tuberculosis* strains are compared to a 'gold standard' that is derived from the results obtained by the majority of the laboratories (judicial criterion). Sensitivity, specificity and reproducibility of susceptibility testing are calculated for each laboratory and for each of the four drugs tested, i.e., INH, RMP, streptomycin (SM), and ethambutol (EMB).⁴⁷

Quality assurance indicators for DST of *M. tuberculosis* in the WHO/IUATLD SRL network ⁴⁷

- **Sensitivity** Ability to detect true resistance
- **Specificity** Ability to detect true susceptibility
- **Efficiency or Accuracy** Ratio between the number of correct results and the total number of results
- **Predictive value for resistance** The rate of true resistance to total resistance
- **Predictive value for susceptibility** The rate of true susceptibility to total susceptibility
- **Reproducibility or Reliability** Intra-laboratory agreement between duplicate cultures expressed as percent agreement

Issues on sample size, identification and transportation of cultures, and analysis (*Bayesian analysis*) of the results, are explained in detail in several publications.^{47–50} The number of SRLs which participated in all consecutive rounds of strain exchange is shown below.

Number of SRLs participating in six rounds of strain exchange for DST

Round 1 (1994)	Round 2 (1995)	Round 3 (1996)	Round 4 (1997)	Round 5 (1998)	Round 6 (1999)
16	18	20	22	22	23

2.3 METHODS OF LABORATORY DIAGNOSIS OF ANTI-TUBERCULOSIS DRUG RESISTANCE

Four DST methods have been standardized and are widely used throughout the world to measure drug resistance of *M. tuberculosis*.^{51–53} In general, participating laboratories used the DST method with which they were most familiar: this was to eliminate variability due to disruption of routine testing through changing to a new testing procedure. The Global Project focuses on resistance to four of the first-line anti-tuberculosis drugs, INH, RMP, EMB and SM.

Methods of laboratory diagnosis of anti-tuberculosis drug resistance used in the Global Project

- Absolute concentration method
- Resistance ratio method
- Proportion method and its variants
- BACTEC 460[®] radiometric method

2.4 REVISED TERMS TO IDENTIFY DRUG RESISTANCE

Since its initiation in 1994 the Global Project had used the terms “*acquired drug resistance*” and “*primary drug resistance*”. For the purpose of this report, and in the light of discussions in several international fora, these terms will not be used any longer. In fact, these terms suggest the exact causative nature of drug resistance, which is rarely possible to assess. For instance, for several reasons patients may not disclose prior TB treatment. If this occurs, the term “*primary drug resistance*” may be used inappropriately, as resistance may have been acquired during the previous concealed treatment. On the other hand, patients who fail treatment may do so because their strain was initially resistant and not because it acquired resistance during treatment. In view of this, the terms “*drug resistance among new cases*” as a proxy of primary resistance, and “*drug resistance among previously treated cases*” as a proxy of acquired resistance, will be used throughout this report. Countries are encouraged to double-check the patient history and thoroughly evaluate medical records and charts to prevent misclassification of previously treated cases as new cases. This will prevent an overestimation of the prevalence of drug resistance among new TB cases.

Definitions of drug resistance

- **Drug resistance among new cases** (formerly: “primary drug resistance”) is the presence of resistant strains of *M. tuberculosis* in a newly diagnosed patient who has never received TB drugs or has received them for less than one month of treatment.
- **Drug resistance among previously treated cases** (formerly “acquired drug resistance”) is that found in a patient who has previously received at least one month of TB therapy.

2.4.1 Drug resistance among new cases (formerly “primary drug resistance”)

Drug resistance among new cases is defined as the presence of resistant strains of *M. tuberculosis* in new TB cases who, in response to direct questioning, deny having had previous anti-tuberculosis treatment or having been treated for more than a month and, in countries where adequate documentation is available, no evidence of such treatment history exists.

2.4.2 Drug resistance among previously treated cases (formerly “acquired drug resistance”)

Patients diagnosed with TB and started on anti-tuberculosis treatment, whose disease is due to bacilli which have developed drug resistance to one or more of the medications used during treatment, are said to have developed “acquired (or secondary) drug resistance”. This can only be demonstrated if the baseline susceptibility of the infecting strain to a given drug was documented before treatment with the specified drug was given.⁵⁴ Such an approach is only possible—and only to some extent—in countries with the resources to perform such determinations and document the results systematically. In most settings, however, documentation of drug susceptibility before the initial treatment is not feasible.

The term “drug resistance in previously treated cases” will thus be used to indicate resistance in TB cases who have already received at least one month of anti-tuberculosis therapy, as documented in the tuberculosis registry, medical records, or by the patient’s account, and who are started on a retreatment regimen. The following categories apply: patients who relapse after having successfully completed treatment in the past; patients who failed treatment; patients who return after treatment default; and chronic patients. These definitions and terms are in line with those described in the WHO Framework for Effective Tuberculosis Control.⁵⁵

2.4.3 Combined prevalence of drug resistance

Combined prevalence of drug resistance is that measured in all cases regardless of prior drug treatment, in a given year. To obtain estimates of the combined prevalence of drug resistance, for geographical settings reporting data from new and previously treated cases separately, we used the same approach as outlined in the first report.^{1,2} For geographical settings conducting surveillance in 100% of their TB patients, we added the data from new and previously treated cases. For geographical settings conducting surveys, regardless of the different sampling schemes for new and previously treated cases, we also combined their separate reports. However, the contribution of drug resistance in previously treated

cases was weighted by the proportion of previously treated cases among all cases registered for treatment in the NTP in the year of the survey, instead of using the proportions of the two subgroups as reported. These proportions were obtained directly from the geographical settings or from reports available to WHO through the NTPs.

2.5 SURVEY AREAS AND SAMPLING STRATEGIES

New surveillance/survey projects presented in this report were carried out between 1996 and 1999 (Table 1). Data on trends are based on geographical settings with at least two data points between 1994 and 1999. Specific details from some of the participant geographical settings follow. As in the first report, England & Wales, Scotland, and Northern Ireland are analysed separately, since they reported their data separately for the years of study. A new study is reported from Italy, a country that was excluded from the global analysis in the first report because only HIV-infected patients had been studied. The results from Henan Province (in China) for 1996 are included in this report, having been put on hold for verification in the first report. Final data from the Thailand survey are presented since the results presented in the first report were preliminary and limited to 131 cases. Final data from Colombia, Guangdong Province (China), Nepal, and Venezuela were not available at the time this report was written; thus, the results included in this report should be considered preliminary.

2.5.1 Target survey areas

For each survey, the target population was made up of all registered smear-positive TB cases in the survey area. In most countries, the survey area was the entire country (Table 2). In Sierra Leone, the survey area excluded some centres *a priori* because of problems primarily related to access (i.e. remote regions, war zones, etc.). Surveys in some large countries, such as China, India, Mexico, the Russian Federation, and South Africa, were restricted to one or more large administrative units (e.g., province, state, oblast). Also, in the Central African Republic, Morocco and Spain, the surveys were limited to the cities of Bangui, Casablanca and Barcelona, respectively. In France, the surveyed area was again composed of selected sentinel sites. Denmark data did not include Greenland and Faroe Islands. In Uganda, the survey only included three of the nine regions of the country. These were the regions assisted by the German Leprosy Relief Association (GLRA).

2.5.2 Sample size and sampling strategies

Table 2 presents sampling methods used by the geographical settings participating in this phase of the Global Project. Sample size calculation for surveys followed the principles outlined in the WHO/IUATLD Guidelines for Surveillance of Drug Resistance in Tuberculosis.^{28,29} Sample size was calculated from the expected prevalence of RMP resistance in new TB cases, or the drug with the lowest prevalence of resistance, estimated from previous studies or based on data available from the NTP. In the absence of previous data, the educated guess of investigators was used.⁵⁶ Annex 1 provides additional details and examples of sampling methodology.^{28,29} Previously treated cases were sampled but no calculation of sample size was made, because of the small proportion of this population in the total pool of TB cases. Thus, sampling of previously treated cases was in most instances limited to the period needed to complete the sample size of new TB cases.

Table 1. WHO/IUATLD Network of Supranational Reference Laboratories and functioning National Reference Laboratories (1996–2000)

Supranational Reference Laboratory	Country or territory	Status	National Reference Laboratory
Queensland Diagnostic and Reference Laboratory for Mycobacterial Diseases, Brisbane, Australia	Australia New Zealand Tamil Nadu, India	Ongoing surveillance Ongoing surveillance Completed survey	The SRL itself TB Reference Laboratory, Green Lane Hospital, Auckland Tuberculosis Research Centre, Madras
INPPAZ - Instituto Panamericano de Proteccion de Alimentos y Zoonosis, Buenos Aires, Argentina	Argentina Chile Cuba Nicaragua Peru Uruguay Venezuela	Ongoing Completed survey Ongoing surveillance Completed survey Completed survey Completed survey Ongoing survey	Administración Nacional de Laboratorios e Institutos de Salud (ANLIS), Buenos Aires Instituto de Salud Pública de Chile, Santiago de Chile Instituto de Medicina Tropical "Pedro Kouri", Havana Ministerio de Salud, Managua Instituto Nacional de Salud, Lima Comision Honoraria para la Lucha Antituberculosa y Enfermedades Prevalentes, Montevideo Laboratorio Nacional de Referencia El Algodonal
Laboratory Centre for Disease Control, Ottawa, Canada	Canada	Ongoing surveillance	The SRL itself
Instituto de Salud Pública de Chile, Chile.	Colombia	Ongoing survey	Instituto Nacional de Salud, Bogota
National Institute of Public Health, Czech Republic	Czech Republic Slovakia	Ongoing survey Ongoing survey	The SRL itself National Institut of TB and Respiratory Diseases, Bratislava
Institut Pasteur, Paris, France	Bangui, Central African Republic France Guinea Morocco New Caledonia Oman Nigeria Ivanovo Oblast, Russian Fed.	Completed survey Ongoing surveillance Completed survey Completed survey Completed survey Completed survey Ongoing survey Ongoing surveillance	Institut Pasteur de Bangui National Reference Centre for the Surveillance of TB, Paris Laboratoire de Reference des Mycobacteries, Conakry Institut Pasteur de Maroc, Casablanca Laboratoire de bacteriologie, Institut Pasteur de Nouvelle Caledonie National Reference TB Laboratory, Darsait Nigerian Institute of Medical Research, Lagos Central Tuberculosis Research Institute, Moscow
National Reference Center for Mycobacteria, Borstel, Germany	Germany Slovenia	Completed survey Ongoing surveillance	The SRL itself University Clinic Respiratory Diseases and Allergy, Laboratory for Mycobacteria, Gornik
Kuratorium Tuberkulose in der Welt E. V., Gauting, Germany	Nepal	Ongoing survey	GENETUP National Tuberculosis Centre and Laboratory, Kathmandu
Armauer-Hansen Institute, Wurtzburg, Germany	Sierra Leone Uganda	Completed survey Completed survey	The SRL itself Central Tuberculosis Laboratory, Kampala
Istituto Superiore di Sanità, Rome, Italy	Italy Albania	Completed survey Planning stage	Istituto Villa Marelli, Milan Institute of TB and Lung Diseases
Research Institute of Tuberculosis, Japan	Cambodia Islamic Republic of Iran Malaysia	Planning stage Completed survey Completed survey	To be determined National Research Institute of Tuberculosis and Lung Disease, Tehran Institute of Respiratory Medicine, Kuala Lumpur

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	Mongolia Philippines Singapore	Ongoing survey Planning stage Completed survey	National Centre for Tuberculosis, Ulaanbaatar To be determined Central Tuberculosis Laboratory, Department of Respiratory Medicine, Ten Tock Seng Hospital, Singapore
Korean Institute of Tuberculosis, Republic of Korea	Republic of Korea Shandong Province, China Hong Kong SAR, China Henan Province, China Guandong Province, China Zhejiang Province, China Thailand	Completed survey Completed survey Completed survey Completed survey Ongoing survey Completed survey Completed survey	The SRL itself Provincial Reference Laboratory, Shandong Tuberculosis Reference Laboratory, Yung Fung Shee Memorial Centre, Hong Kong SAR Henan Anti-tuberculosis Institute, Henan Provincial Reference Laboratory, Guandong Provincial Reference Laboratory, Zhejiang Laboratory of Tuberculosis Division (DCDC), Ministry of Health, Bangkok
National Institute of Public Health and Environmental Protection (RIVM), Bilthoven, Netherlands	Netherlands Poland	Ongoing surveillance Completed survey	Various laboratories under coordination by SRL itself Microbiology Department, National TB and Research Institute, Warsaw
National Institute of Public Health, Norway (Linked to Swedish Institute for Infectious Disease Control, Sweden)	Mozambique	Completed survey	National Reference Laboratory of Mozambique, Maputo
Servicio de Microbiología, Hospitales Universitaris Vall d'Hebron, Barcelona, Spain	Barcelona	Completed survey	The SRL itself
Medical Research Council National TB Research Programme, South Africa	Mpumalanga Province, South Africa Nationwide, South Africa Zambia, South Africa	Completed survey Planning stage Planning stage	The SRL itself The SRL itself Lusaka Chest Disease Laborator, Lusaka
Swedish Institute for Infectious Disease Control (SIIDC), Stockholm, Sweden	Denmark Estonia Finland Latvia Norway Sweden	Ongoing surveillance Ongoing surveillance Ongoing surveillance Ongoing surveillance Ongoing surveillance Ongoing surveillance	Statens Serum Institute, Copenhagen Tuberculosis Reference Laboratory, Tartu Mycobacterial Reference Laboratory, National Public Health Institute, Turku State Centre of Tuberculosis and Lung Diseases, Riga National Institute of Public Health, Oslo The SRL itself
Public Health Laboratory Service (PHLS) Mycobacterium Reference Unit, London, UK	England & Wales Northern Ireland Scotland Belgium Gambia Israel Switzerland	Ongoing surveillance Ongoing surveillance Ongoing surveillance Ongoing surveillance Ongoing survey Ongoing surveillance Ongoing surveillance	The SRL itself Northern Ireland Reference Laboratory, Belfast Scottish Mycobacteria Reference Laboratory, Edinburgh Institut Pasteur de Bruxelles MRC Laboratory, Banjul Public Health Laboratory, Tel Aviv National Center for Mycobacteria, University of Zurich
Centers for Disease Control and Prevention (CDC), Atlanta, United States of America	United States of America Botswana Mexico Puerto Rico	Ongoing surveillance Completed survey Completed survey Ongoing surveillance	Multiple laboratories following national standards National Health Laboratory, Gaborone Instituto Nacional de Diagnóstico y Referencia Epidemiológicos (INDRE), Mexico City Laboratorio Central de Tuberculosis, San Juan
Massachusetts State Laboratory, Boston, United States of America	Tomsk Oblast, Russian Fed.	Completed survey	Central Tuberculosis Research Institute, Moscow

Table 2. Sampling methodology in the Global Project

COUNTRY	REPORT YEAR	PROJECT STATUS	TOTAL DURATION (MONTHS)	TARGET AREA	SAMPLING METHOD	FRACTION SAMPLED (%)*
Australia	1996	Ongoing surveillance	12	Country-wide	All cases	100
Belgium	1997	Ongoing surveillance	12	Country-wide	All cases	100
Botswana	1999	Completed survey	22	Country-wide	Random	10
Canada	1997	Ongoing surveillance	12	Country-wide	All cases	100
Central African Republic (Bangui)	1998	Completed survey	3	City-wide	All cases	100
Chile	1997	Completed survey	6	Country-wide	Proportionate cluster	50
China (Henan Province)	1996	Completed survey	9	Province	Proportionate cluster	11
China (Guangdong Province)	1998–99	Ongoing survey	12	Province	Proportionate cluster	5
China (Hong Kong SAR **)	1996	Ongoing surveillance	12	Province	All cases	100
China (Shandong Province)	1997	Completed survey	12	Province	Proportionate cluster	5
China (Zhejiang Province)	1998–99	Ongoing survey	12	Province	Proportionate cluster	4
Colombia	1999	Ongoing survey	12	Country-wide	Cluster	10
Cuba	1998	Ongoing surveillance	12	Country-wide	Proportionate cluster	33
Czech Republic	1999	Completed survey	12	Country-wide	All cases	100
Denmark	1998	Ongoing surveillance	12	Country-wide	All cases	100
England & Wales	1997	Ongoing surveillance	12	Country-wide	All cases	100
Estonia	1998	Ongoing surveillance	12	Country-wide	All cases	100
Finland	1997	Ongoing surveillance	12	Country-wide	All cases	100
France	1997	Ongoing surveillance	12	Sentinel sites	All cases	100
Germany	1998	Completed survey	12	Sentinel sites	Random	66
Guinea	1998	Completed survey	10	Sentinel sites	Random	15
India (Tamil Nadu State)	1997	Completed survey	3	State	Proportionate cluster	100
Islamic Republic of Iran	1997–98	Completed survey	12	Country-wide	Random	10
Israel	1998	Ongoing surveillance	12	Country-wide	All cases	100
Italy	1998–99	Completed survey	12	Half of the country	Cluster	23
Latvia	1998	Ongoing surveillance	3	Country-wide	All cases	100
Malaysia	1996–97	Completed survey	17	Peninsular Malaysia	Cluster	9
Mexico (Baja California, Oaxaca and Sinaloa)	1997	Completed survey	7	3 of 31 States	All cases	50
Morocco (Casablanca)	1997–98	Completed survey	6	City-wide	Cluster	25
Mozambique	1998–99	Completed survey	9	Country-wide	Proportionate cluster	7
Nepal	1999	Ongoing survey	6	Country-wide	All cases	100
Netherlands	1996	Ongoing surveillance	12	Country-wide	All cases	100
New Caledonia	1995–96	Completed survey	21	Country-wide	All cases	100
New Zealand	1997	Ongoing surveillance	12	Country-wide	All cases	100
Nicaragua	1997–98	Completed survey	20	Country-wide	Proportionate cluster	20
Northern Ireland	1997	Ongoing surveillance	12	Country-wide	All cases	100

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COUNTRY	REPORT YEAR	PROJECT STATUS	TOTAL DURATION (MONTHS)	TARGET AREA	SAMPLING METHOD	FRACTION SAMPLED (%) [*]
Norway	1996	Ongoing surveillance	12	Country-wide	All cases	100
Oman	1998–99	Completed survey	12	Country-wide	All cases	100
Peru	1999	Completed survey	8	Country-wide	Proportionate cluster	7
Poland	1996–97	Completed survey	12	Country-wide	All cases	100
Puerto Rico	1997	Ongoing surveillance	12	Country-wide	All cases	100
Republic of Korea	1998–99	Completed survey	4	Country-wide	All cases	100
Russian Fed. (Tomsk Oblast)	1998–99	Complete survey	12	Province	All cases	100
Russian Fed. (Ivanovo Oblast)	1998	Ongoing surveillance	12	Province	All cases	100
Scotland	1997	Ongoing surveillance	12	Country-wide	All cases	100
Sierra Leone	1997	Completed survey	6	Nearly country-wide	Random	15
Singapore	1996	Completed survey	12	Country-wide	All cases	100
Slovakia	1998	Completed survey	12	Country-wide	All cases	100
Slovenia	1997	Ongoing surveillance	12	Country-wide	All cases	100
South Africa (Mpumalanga Prov.)	1997	Completed survey	6	Province	Proportionate cluster	43
Spain (Barcelona)	1997–98	Completed survey	24	City-wide	Cluster	59
Sweden	1997	Ongoing surveillance	12	Country-wide	All cases	100
Switzerland	1997	Ongoing surveillance	12	Country-wide	All cases	100
Thailand	1996–97	Completed survey	12	Country-wide	Proportionate cluster	13
Uganda (GLRA supported zones ^{***})	1996–97	Completed survey	18	3 Zones	Cluster	3
United States of America	1997	Ongoing surveillance	12	Country-wide	All cases	100
Uruguay	1997	Completed survey	12	Country-wide	All cases	100
Venezuela	1998	Ongoing survey	6	Country-wide	Proportionate cluster	100

- * Sampled fraction of all eligible tuberculosis patients in the target area
- ** Special administrative region
- *** German Leprosy Relief Association

Sampling strategies for Drug Resistance Surveillance

- Country-wide, ongoing surveillance of the population
- Surveys with 100% sampling during a specified time period
- Surveys with a simple random sample of TB patients
- Surveys of randomly selected clusters (i.e. diagnostic centres) of patients
- Surveys with cluster sampling proportional to the population

2.6 BACTERIOLOGICAL METHODS USED IN SURVEYS/SURVEILLANCE

The majority of laboratories used Löwenstein-Jensen (L-J) culture medium. A few others used the Ogawa culture medium. Identification of the strains was based on the niacin production test, the nitrate reduction test, the aminobenzoic acid (500 mg/l) and the thiophene carboxylic acid (2 mg/l) resistance test. Some countries use also hybridization probes. Species other than the pathogenic species of the *M. tuberculosis* complex were excluded from analysis.

Drug resistance tests were performed using the economic variant of the proportion method on L-J medium,⁵¹ the absolute concentration method, the resistance ratio method, or the radiometric Bactec 460 method (Table 3). The proportion method was the most frequently used (62% of the participating settings) in this phase of the Global Project. Resistance was expressed as the percentage of colonies that grew on critical concentrations of the drugs (i.e. 0.2 mg/l for INH, 2 mg/l for EMB, 4 mg/l for dihydrostreptomycin sulphate and 40 mg/l for RMP when L-J medium was used). The criterion for resistance to a particular drug was growth of 1% of the population on medium containing the critical concentration. The results of the tests were then recorded on standardized laboratory forms (see Annex 1), copies of which were collected by each national coordinator and reported to WHO.

Proficiency testing was conducted between participant settings and the corresponding SRLs, as in the first phase of the Global Project.^{1,2} Table 3 lists the number of specimens exchanged and the overall agreement (i.e. concordance of results) between national reference laboratories (NRLs) of participant geographical settings and SRLs for the four drugs evaluated. In most cases, significant discrepancies were clarified before implementing the survey.

2.7 COLLECTION OF DATA

All newly registered patients with smear-positive TB were eligible for inclusion, including children, foreign-born persons, hospitalized patients, and those with known HIV co-infection. As in the previous phase of the Global Project, HIV testing was not a systematic component of these surveys. Geographical settings that performed HIV testing as part of the survey were advised to follow international guidelines on counselling and confidentiality.⁵⁷ Reports from Australia, Belgium, Canada, and Israel did not distinguish between resistance in new and previously treated cases, and only the combined prevalence of drug resistance is presented and analysed. Belgium reported resistance data for INH and RMP, since testing for EMB and SM was not systematically performed.

In several surveys (Benin, Henan and Shandong Provinces in China, Morocco, New Caledonia, Oman, Peru, and Uganda), re-interview and double-checking of the patients' his-

tories was undertaken to reduce the possibility of misclassification of previously treated cases as new cases. In this phase of the Global Project, version 2 of the WHO software “Surveillance of Drug Resistance in Tuberculosis” (SDRTB 2.0) was used for data entry, management and analysis at the local level. Most industrialized countries use their own software for surveillance. Aggregated (all geographical settings) and individual (selected geographical settings) data were provided to WHO for global analysis. Demographics, including sex and age, prior history of TB therapy and HIV test results, were the variables recommended for collection.

2.7.1 Data collection by place of origin

For the first time, data according to the place of origin of patients were requested in this phase of the Global Project. A simple data collection form was designed (Annex 1) for projects to provide information on the magnitude of any drug resistance and MDR-TB according to indigenous and foreign-born populations. Responses to this request were mainly from low TB incidence countries.

2.7.2 Statistical analysis

Descriptive statistics of the study population and bivariate analyses were calculated in Epi-Info 6 and SPSS/Windows 7.5.2. Median values were calculated for the prevalence of drug resistance in new cases, previously treated cases, or combined, for individual drugs and pertinent combinations. In addition to median values, mean values were weighted by the estimated number of smear-positive cases in each geographical setting using the SPSS weighting procedure. This procedure weights cases for analysis based on the value of the weight variable. The distribution of the prevalence of the different patterns of drug resistance was illustrated using box-plots, which display the median, quartiles and outliers. The latest data point available for each geographical setting was used in box-plots, maps, and figures.

Estimation of coverage of the Global Project was done using TB cases reported to WHO,⁵⁸⁻⁶² and population figures for year 1997 as estimated by the United Nations Population Division “*World Population Prospects; 1998 Revision*”. For geographical settings reporting more than two data points, only the latest one was used for these calculations. Also, for surveys carried out in administrative units of large countries (states, provinces, oblasts) only notified TB cases and population of these administrative units were used. It is important to acknowledge that estimates regarding coverage are approximations. They should be interpreted with caution because of the changes in population and in the incidence of TB over time. Nevertheless, while there is a certain degree of uncertainty about these estimates, TB incidence and population figures do not change grossly between years.

Standard chi-square and Fisher’s exact two-tailed test were used to compare differences between indigenous and foreign-born cases with TB (new and previously treated) for any drug resistance and MDR-TB.

2.8 TRENDS IN DRUG RESISTANCE SURVEILLANCE

In order to assess current trends in anti-tuberculosis drug resistance prevalence, geographical settings surveyed in the first phase of the Global Project were encouraged to repeat the surveys or to provide new data if a surveillance system was in place.

Table 3. Laboratory and performance at each of the NRLs in the Global Project

COUNTRY	CULTURE METHOD	DST METHOD	PT* STRAINS	NRL/SRL AGREEMENT (%)	Specificity for RMP DST	PATIENTS TESTED
Australia	Löwenstein-Jensen & Bactec	Bactec	20	100	100	750
Belgium	Löwenstein-Jensen & Bactec	Proportion	20	90	100	791
Botswana	Löwenstein-Jensen	Resistance ratio	18	94	100	783
Canada	Various	Bactec	20	98	100	1593
Central African Republic (Bangui)	Löwenstein-Jensen	Proportion	24	97	100	497
Chile	Löwenstein-Jensen	Proportion	20	100	100	881
China (Henan Province)	Löwenstein-Jensen	Proportion	292	91	92	1372
China (Guangdong Province)	Löwenstein-Jensen	Resistance ratio	30	96	97	524
China (Hong Kong SAR **)	Löwenstein-Jensen	Absolute concentration	30	94	100	5207
China (Shandong Province)	Löwenstein-Jensen	Proportion	30	96	100	1229
China (Zhejiang Province)	Löwenstein-Jensen	Proportion	30	95	100	942
Colombia	Ogawa	Proportion	20	97	100	201
Cuba	Löwenstein-Jensen	Proportion	60	98	100	327
Czech Republic	Löwenstein-Jensen & others	Proportion	20	98	100	363
Denmark	Löwenstein-Jensen & Bactec	Bactec	20	98	100	444
England & Wales	Löwenstein-Jensen & Bactec	Resistance ratio	20	96	100	3242
Estonia	Löwenstein-Jensen & Bactec	Proportion + Bactec	65	90	100	459
Finland	Löwenstein-Jensen	Proportion	20	95	100	412
France	Löwenstein-Jensen & Bactec	Proportion	20	100	100	852
Germany	Löwenstein-Jensen & Bactec	Proportion + Bactec	20	98	100	1711
Guinea	Löwenstein-Jensen	Proportion	26	95	100	571
India (Tamil Nadu State)	Löwenstein-Jensen	Resistance ratio	20	99	100	400
Islamic Republic of Iran	Löwenstein-Jensen	Proportion	260	94	100	722
Israel	Löwenstein-Jensen	Resistance ratio	20	95	100	307
Italy	Löwenstein-Jensen	Proportion + Bactec	20	98	100	810
Latvia	Löwenstein-Jensen	Absolute concentration	35	95	100	1013
Malaysia	Ogawa	Absolute concentration	84	98	100	1017
Mexico (Baja California, Oaxaca and Sinaloa)	Löwenstein-Jensen	Bactec	20	98	100	441
Morocco (Casablanca)***	Löwenstein-Jensen	Proportion	510	100	100	510
Mozambique	Löwenstein-Jensen	Proportion	70	90	100	1150
Nepal	Löwenstein-Jensen	Proportion	77	92	97	131
Netherlands	Various	Absolute concentration	20	91	100	1214
New Caledonia	Löwenstein-Jensen	Proportion	8	95	100	105
New Zealand	Löwenstein-Jensen & Bactec	Bactec	20	98	100	200
Nicaragua	Löwenstein-Jensen	Proportion	9	100	100	564
Northern Ireland	Löwenstein-Jensen & Bactec	Resistance ratio	20	100	100	41

... continued

COUNTRY	CULTURE METHOD	DST METHOD	PT* STRAINS	NRL/SRL AGREEMENT (%)	Specificity for RMP DST	PATIENTS TESTED
Norway	Löwenstein-Jensen	Bactec	20	98	100	282
Oman	Löwenstein-Jensen	Proportion	9	91	100	133
Peru	Löwenstein-Jensen	Proportion	16	100	100	2139
Poland	Löwenstein-Jensen & Bactec	Proportion	40	96	100	3970
Puerto Rico	Bactec	Proportion & Bactec	20	92	100	172
Republic of Korea	Löwenstein-Jensen	Proportion	20	97	100	2653
Russian Fed. (Tomsk Oblast)	Löwenstein-Jensen	Absolute concentration	121	82	96	649
Russian Fed. (Ivanovo Oblast)	Löwenstein-Jensen	Absolute concentration	39	95	97	276
Scotland	Bactec	Bactec	17	100	100	307
Sierra Leone	Löwenstein-Jensen	Proportion	130	95	100	130
Singapore	Löwenstein-Jensen & Bactec	Bactec	20	99	100	1131
Slovakia	Löwenstein-Jensen	Proportion	20	94	90	746
Slovenia	Löwenstein-Jensen	Proportion	10	100	100	326
South Africa (Mpumalanga Prov.)	Löwenstein-Jensen	Proportion	20	89	91	761
Spain (Barcelona)	Löwenstein-Jensen & Bactec	Proportion	20	95	100	384
Sweden	Löwenstein-Jensen & Bactec	Bactec	20	94	100	380
Switzerland	Various	Various	20	99	100	362
Thailand	Löwenstein-Jensen	Proportion	30	91	97	1137
Uganda (GLRA supported zones ****)	Löwenstein-Jensen	Proportion	121	98	100	419
United States of America	Various	Various	20	92	100	12675
Uruguay	Löwenstein-Jensen	Proportion	20	98	100	500
Venezuela	Löwenstein-Jensen	Proportion	13	90	100	245

* Number of strains exchanged between NRL and SRL for proficiency testing (PT)

** Special administrative region

*** All strains collected in Morocco were re-tested at the SRL due to the high discordant results on the quality control exercise. Values for agreement and specificity are those of SRL

**** German Leprosy Relief Association

Twenty-eight geographical settings provided two or more data points between 1994 and 1999, following the same surveillance/survey methodology.

Geographical settings for which two or more data points in drug-resistant TB were analysed

- **AFRICA** Sierra Leone, Botswana
- **AMERICAS** Canada, Chile, Cuba, Peru, Puerto Rico, United States of America
- **EUROPE** Barcelona (Spain), Belgium, Czech Republic, Denmark, England & Wales, Estonia, Finland, France, Germany, Ivanovo Oblast (Russian Federation), Latvia, Netherlands, Northern Ireland, Scotland, Sweden, Switzerland
- **ASIA** Nepal, Republic of Korea
- **OCEANIA** Australia, New Zealand

2.8.1 Statistical analysis of trends

Analysis focused on drug resistance found in new cases, previously treated cases and in the combined prevalence of drug resistance. The following patterns of drug resistance were highlighted: any drug resistance, MDR-TB, any INH resistance, and any RMP resistance. Chi-square standard test was used for the comparison of two data points (proportions), and chi-square for trends was used for the comparison of three or more data points.

2.9 ECOLOGICAL ANALYSIS OF DRUG RESISTANCE AND NATIONAL TUBERCULOSIS PROGRAMME CHARACTERISTICS

In the first phase of the Global Report the prevalence of drug resistance in each geographical setting was correlated with characteristics of the NTP.¹ One of the limitations of this analysis was the lack of sufficient statistical power to perform sub-analyses. The greater number of geographical settings available in the two phases of the Global Project combined ($n = 72$) increased substantially the statistical power to detect differences. Therefore, a new ecological analysis was done to compare aggregated data on drug resistance at group level (by geographical setting) with indicators of TB control and development.⁶³⁻⁶⁵ For geographical settings with more than two data points, the data collected in the most recent year of surveillance were used for this analysis.

2.9.1 Variables included in the ecological analysis

Outcome (dependent) variables examined in this report included:

- proportion of any drug resistance among new cases
- proportion of MDR-TB among new cases.

Potential explanatory (independent) variables examined in this report included:

- notified TB incidence rate;⁵⁸⁻⁶²
- proportion of all cases presenting to treatment that were previously treated [i.e. failures, patients returning after default, relapses and chronic cases];
- treatment success;

- proportion of TB patients under treatment with SCC;
- proportion of TB patients under DOT;
- proportion of TB patients treated with fixed-dose combination (FDC) tablets;
- gross national product (GNP) per capita income;
- estimated proportion of TB patients infected with HIV.

Median values of any drug resistance and MDR-TB among new cases were estimated according to WHO geographical regions* and to WHO/ DOTS control category.**

These data were obtained from the participating countries through a standardized questionnaire (see Annex 1) and from the publications “*Global Tuberculosis Control*” 1997, 1998, and 1999.⁵⁸⁻⁶⁰

Data reported to WHO in 1994, 1995, and 1996 were used to derive a three-year average treatment success in each participant setting. Patients registered in DOTS and non-DOTS areas were added to obtain the total number of patients registered in a given setting/area. Then, patients who were not evaluated (unknown outcome) were excluded. If the total number of patients evaluated was less than 10% of all smear-positive cases registered for the setting, the data were excluded since they were judged not to be representative.

2.9.2 Statistical analysis of ecological data

Initially, associations between continuous predictors and drug resistance values were evaluated by the Spearman’s correlation coefficient (r_s). Scatterplots were generated to illustrate selected correlations. Weighted logistic regression modelling was used to explore the contribution of different variables to the prevalence of any drug resistance and of MDR-TB (separate modelling for both response variables) in new TB cases. Since the prevalence of any drug resistance and of MDR-TB take the form of proportions in each geographical setting, these variables are strictly bounded (i.e., no percentage > 100% or < 0%) and thus follow a binomial distribution (i.e. number of TB cases with any drug resistance out of the total number of cases tested). Therefore, to ensure linearity we used the *logit link function* to regress the explanatory or independent variables on the response or dependent variable. Modelling was weighted using the individual sample sizes (of each geographical setting) as weights in order not to lose information of the size of the sample from which such proportions were estimated. If only the percentages of any drug resistance or MDR-TB are used as dependent variables, sample sizes are not taken into account.⁶⁶

Each variable was modelled by univariate logistic regression and plotted against the response variable in order to explore its individual contribution as well as departures from normality and variance instability. As a result, three variables were transformed: GNP per capita income and TB incidence into the logarithmic scale; and the proportion of patients under SCC into the arcsine or angular transformation.

Multivariate weighted logistic regression modelling was used to obtain adjusted estimates. Several models were explored using the backward elimination method in order to find the model that best fitted the data. To assess the goodness of fit of the models and account for over-dispersion (i.e., random variation), we divided the Pearson χ^2 value by the

* AFR for sub-Saharan Africa, AMR for the Americas, EMR for the Eastern Mediterranean region, EUR for Europe, SEAR for South-East Asia, and WPR for the Western Pacific Region

** Category 0 for countries not reporting to WHO, category 1 for countries not implementing DOTS and TB notification rate >10/100 000, category 2 for countries implementing DOTS in <10% of the population, category 3 for countries implementing DOTS in 10%–90% of the population, category 4 for countries implementing DOTS in >90% of the population, and category 5 for countries not using DOTS and TB notification rates < 10/100 000

degrees of freedom and compared the resulting scaled deviances for terms in the model using an F -Test instead of χ^2 (as in conventional ANOVA).

2.10 EFFECT OF DEMOGRAPHICS AND OTHER INDIVIDUAL PATIENT'S DATA ON DRUG RESISTANCE

2.10.1 Participant countries and procedures

Population-based patient data from 11 geographical settings, surveyed within the WHO/IUATLD Global Project between 1994 and 1998, were used to assess the effects of demographic characteristics (age, sex), prior history of anti-TB treatment, and HIV on the dynamics of drug-resistant TB. Data were available from Bolivia, Dominican Republic, Republic of Korea, Lesotho, Nepal, Peru, Portugal, Sierra Leone, Swaziland, Barcelona (Spain), and Shandong Province (China). These geographical settings provided (before a pre-established deadline) detailed individual-level data on the patients enrolled in their drug resistance studies. Some other geographical settings also provided individual data. However, since these data arrived late, they could not be included in this analysis.

Patients also reported the number of past episodes of treatment and number of months of treatment during each episode. This information allowed the calculation of the total time a patient was on prior TB treatment. In addition, clinical and laboratory records were reviewed and abstracted to detect previous treatment for TB.

At each site, data were entered into the WHO software for drug resistance, SDRTB-2. Data were then sent to WHO for review and merging into a global database. Inconsistencies and apparent data entry errors were discussed and clarified with the survey coordinators of each geographical setting when necessary. Since SDRTB-2 allowed the investigators to adapt the original entries to the local conditions, including translation to languages other than English, for the purpose of this analysis electronic data provided in other languages were not used.

2.10.2 Statistical analysis of determinants of anti-tuberculosis drug resistance

Statistical analysis was performed using STATA (*Stata statistical software. Release 5.0. College Station, Texas: Stata Corporation, 1997*). Simple proportions and 95% confidence intervals (95% CI) were calculated; differences between proportions were assessed by standard chi-square; differences between means of continuous variables were assessed by Student's t -test. Separate univariate and multivariate logistic regression analyses were performed to determine variables associated with resistance to one or more drugs and with MDR-TB. These analyses were performed for all available individuals ($n = 9\ 615$), as well as for a subset of individuals for whom information was available on prior HIV test results ($n = 463$), in order to assess the association between HIV and drug resistance. Odds ratios (OR) and 95% CI were calculated to measure the association between variables at the univariate and multivariate level.