Surveillance of Drug-Resistant Tuberculosis in South-East Asia

*Report of an Intercountry Training Course
Bangalore, India, 21-25 June 2004*

WHO Project: ICP TUB 001

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CONTENTS

1.   INTRODUCTION

Tuberculosis (TB) is the biggest killer of young adults, the age at which they are economically most productive. Nine million new cases of TB occur every year. Two million people die annually of TB and that makes it the biggest killer after HIV. Almost 38% of the global TB burden is in the South-East Asia Region where annually 750 000 deaths occur due to TB, or 1500 everyday, or one every minute. Five of the 22 high burden countries are in the SEA Region. In 1993, WHO declared TB to be a global health emergency and a war against this scourge was initiated through the DOTS (Directly observed treatment, short course) strategy. DOTS and resistance in *Mycobacterium tuberculosis* have a relationship of cause and effect. Poorly implemented DOTS gives rise to greater resistance in tubercle bacilli and prevalence of resistant mycobacteria warrants management through DOTS plus, which is expensive, of longer duration and employs toxic drugs.

In spite of the success of DOTS, TB control programmes are faced with two major problems, HIV and drug resistance. Of the 6 million people living with HIV in the Region, 2.5 million are infected with TB. Nearly 60% of AIDS cases develop TB indicating that the latter is the most common life-threatening opportunistic infection associated with HIV. The occurrence of resistance in these cases will spell a certain and quick death for them. All the five high TB burden countries in the Region, with the exception of Bangladesh, are considered high burden for HIV too. This increases the incidence of co-infection of two major lethal infections.

One of the aims of ensuring effective management of tuberculosis is to minimize the development of drug resistance. Surveillance of anti-TB drug resistance is therefore an essential tool for monitoring the effectiveness of TB control programmes and improving national and global TB control efforts. Drug sensitivity tests for monitoring resistance have been developed and standardized.

In 1994, WHO joined forces with International Union Against Tuberculosis and Lung Diseases (IUATLD) and launched the Global Alliance on Antituberculosis Drug Resistance Surveillance. The aims of the global

project are to measure the prevalence and monitor the trend of anti-TB drug resistance worldwide using a standardized methodology and to study the correlation between the level of the drug resistance and treatment policies in different countries. A global surveillance network for MDR-TB is being developed by WHO headquarters and at present among the countries of the Region, only India, Thailand and Nepal are participating in this. Apart from these three countries, Indonesia, Bangladesh and Myanmar have initiated studies on drug resistance in TB. WHO has supported these activities in past three years.

The need was felt for a review of these activities, results obtained as well as reorientation on the use of correct laboratory techniques and sampling methodology along with future direction for the surveillance activities. Accordingly, an intercountry training course was organized at the National Tuberculosis Institute (NTI), Bangalore, India from 21 to 25 June 2004. It was attended by 18 participants from Bangladesh, India, Indonesia, Myanmar, Nepal and Thailand. The list of participants is at Annex 1 and the programme of work at Annex 2.

2. OBJECTIVES

The following were the objectives of the training course:

(1) To review the status of drug resistance in *Mycobacterium tuberculosis* in the countries of the SEA Region;

(2) To discuss the techniques including sampling methodologies being followed in the Region in undertaking community-based drug resistance surveillance in tuberculosis;

(3) To demonstrate resistance determination techniques with emphasis on quality assurance;

(4) To formulate a mechanism for networking and sharing of data with programme managers, and

(5) To develop a country-specific draft plan of action on drug surveillance.

3. INAUGURAL SESSION

The training course was inaugurated by Dr Prahlad Kumar, Director, National Tuberculosis Institute, Bangalore. He emphasized the importance of resistance in the efficient management of any tuberculosis control programme. Dr Kumar also described the role played by NTI in developing various strategies in TB control. Earlier the participants were welcomed by Dr V.H.Balasangameshwara, Head of the Mycobacteriology Laboratory of the Institute. Dr Rajesh Bhatia, Short-term Professional, SEARO, presented the objectives and expected outcome of the workshop. He also deliberated upon the role of WHO in advocating the strengthening of laboratories in drug resistance surveillance and promoting quality in these areas.

4. THE WORKSHOP

4.1 Drug Resistance in Tuberculosis in the SEA Region

Dr C.Paramasivan, Deputy Director, Tuberculosis Research Centre (TRC), Chennai gave an overview of the global problem of resistance in tuberculosis and shared the results of the third round of global disease surveillance. Drug resistance in previously not treated individuals (primary resistance) in three countries of the Region, viz Thailand, Nepal and India, ranged between 0.9% and 2.8%. The figures for India do not denote national figures since surveillance was undertaken in only three selected sites.

Bangladesh

The estimated new cases of smear-positive tuberculosis in Bangladesh are 105 per 100 000 population per year. A cure rate of 84% of the TB patients, against the target of 85%, and a case detection rate of 38% against the target of at least 70% of new smear-positive patients by the end of 2005 have been achieved. Bangladesh has adopted the WHO recommended DOTS strategy since 1993 to control TB and prevent MDR TB since 1993. It covers 98% of the total population. No national data are available for the prevalence of drug resistance tuberculosis at present. To increase CDR, intensified collaboration with the private sector, working places and prisons is necessary.

India

Drug resistance in new smear-positive pulmonary tuberculosis is common in India and varies widely from area to area. Data from TRC have shown a gradual increase in the prevalence of resistance to anti-tuberculosis drugs. For isoniazid, resistance has ranged from 3% to 17% and for streptomycin from 3% to 13%. Resistance to rifampicin started appearing in the 1990s and remains around 1%. Resistance to more than one drug has been observed and ranges from 0 to 7% for isoniazid and streptomycin $\leq$1% for other combinations. In a recent survey conducted in a rural district in Maharashtra, Wardha, the level of MDR TB among new cases was only 0.5%.

Drug resistance in previously treated pulmonary tuberculosis cases is higher than drug resistance in new cases. A study conducted by TRC in a district in Tamil Nadu, found an increase in the frequency of acquired drug resistance with 67% resistance to isoniazid, 26% to streptomycin and 12% to rifampicin and 6% to both isoniazid and rifampicin. Similar results have been reported from Delhi and Gujarat. Drug resistance in previously treated cases in Chennai was 63%, out of which 23.5% were resistant to single drug and 39.5% resistant to more than one drug.

Multi-drug resistance (MDR) in India is very low and ranges from 0 to 6%. In a recent report from Chennai on the prevalence of MDR-TB among patients undergoing treatment for varying periods of time in four TB sanatoria attached to four district tuberculosis centres in Tamil Nadu showed 20.3% to be harbouring multidrug-resistant strains. The majority of these patients had irregular and interrupted treatment owing to the non-availability of drugs.

Indonesia

Indonesia has implemented the DOTS strategy since 1995. A cure rate of more than 85% has been achieved. By the end 2003 the DOTS strategy has covered around 90% of existing health centres; however, most of the hospitals and the private health sector have not yet adopted the DOTS strategy. The case notification rate is around 40%, while it is estimated that at least 70% of the predicted new smear-positive TB cases are detected.

No countrywide data are currently available on resistance in TB. Some small-scale surveys in Jakarta have shown MDR levels of more than 4% in previously untreated cases, but it remains to be determined whether this

situation prevails throughout the country. A protocol on MDR surveillance following the guidelines for WHO's global monitoring project has been developed and will be implemented in 2004-2005. In 2004 it is proposed to start drug resistance surveillance in East Java Province. This is expected to act as a surveillance model for other provinces.

Myanmar

During 2003, 77214 cases of TB were notified in the country with a notification rate of 154/100 000. The percentage of sputum smear-positive cases in new pulmonary TB cases is 51.3%. The estimated HIV-TB co infection was found to be 4.5% in a survey conducted during 1995-1997. MDR TB among new TB patients in an institution-based study during 1994-1995 was 1.25 %. In 2003, MDR TB among new TB patients was found to be 2.9%.

Nepal

Infection with TB is very common in Nepal. About 60% of the economically active adult population is infected with TB. The annual risk of tuberculosis infection (ARTI] is estimated to be 1.72%. It is also estimated that about 44 000 people develop tuberculosis annually. Nearly half of them (20 000) are sputum-positive for acid fast bacilli (AFB) and about 6,000-8,000 people die from tuberculosis every year. In 2002, 2.4% of TB patients were recorded to have HIV infection. Surveys on drug resistance show that 1.33% of new tuberculosis patients harbour MDR-TB. However, a steady decline has been observed in the rate of MDR-TB, from 3.7% in 1998 to 1.33 in 2001/2002. Surveillance from 1996 to 2001 showed that 41% of the pretreatment patients are resistant to at least one drug. Further, resistance increased from 9.8% in 1996-97 to 13.2% in 1998-99 but decreased to 11.0% in 2001-02. MDR-TB resistance was 1.2% in 1996-97, 3.7% in 1998-99 and 1.3% in 2001-02 among new cases.

Thailand

The National Tuberculosis programme (NTP) in Thailand was established in 1949. TB control was implemented as an integral part of the existing general health services as a permanent programme, aiming to provide countrywide service that is accessible to the population. The Directly Observed Treatment Short-Course (DOTS) was adopted in 1996, and countrywide DOTS coverage achieved in 2001.

In 2003, based on a population size of 63 million, the estimated total incidence for Thailand was reported as 137 per 100,000 population; sputum smear-positive TB cases numbered 38 000, the total number of TB cases was 86 000; the number of deaths from TB was 13 000, and the overall case notification rate was 60 per 100 000 population. Treatment success rate and retreatment rates were 77% and 4.95% respectively and the estimate of HIV-positive TB cases was 7.12%.

Drug resistance surveillance (DRS) was performed in 1997 and 2001. Randomly selected proportionate cluster sampling of patients was adopted countrywide. In 2001, DRS covered 66 clusters from 64 diagnostic centres in 55 of 75 provinces. About 1 677 cases from among new patients with previous treatment were diagnosed to be sputum smear-positive PTB, put on DOTS and cultures isolated from them were subjected to proportional method of susceptibility testing to four drugs, namely rifampicin, isoniazid, streptomycin and ethambutol.

The trend of drug resistance-tuberculosis has shown that MDR TB has decreased, from 2.02% in 1997 to 0.9% in 2003. Among the previously treated patients MDR-TB was shown in 20.3% cases.

4.2   Sampling Techniques for Undertaking Surveillance

Mr K.P. Unnikrishnan, Chief Statistician, NTI, described the importance of proper sampling for drug resistance surveillance, various techniques that are recommended by WHO and the basis of each of these. It was emphasized that the sampling universe for a survey on the prevalence of anti-TB drug resistance should include all newly-registered sputum smear-positive TB patients in the country. The calculation of an appropriate sample size should be based on the following:

> ➢ The total number of new sputum smear-positive cases detected in the previous year in the country or in the geographical setting to be studied;

> ➢ Expected prevalence of resistance to rifampicin from available data (in the absence of available data, the best guess of investigators should be used);

> ➢ Precision should be between 1 and 2%, while ensuring that obtaining the calculated sample is logistically feasible;

> ➢ A confidence interval of 95% should be used for estimated prevalence.

Different sampling strategies can be adopted to select a representative sample of TB patients. In order to select a representative group of newly registered patients, a randomization step is essential. Some of the most useful sampling strategies that were discussed were:

100% sampling of diagnostic centres

This sampling method is most suitable for small countries with relatively small numbers of TB diagnostic units, good infrastructure, and facilities for transportation of samples from all.

Cluster sampling

Cluster sampling methods are best used in situations where it is logistically difficult to cover the entire area of the country and where the number of TB diagnostic centres is high. With this design, centres are randomly selected, and all sputum smear-positive patients newly registered at these selected centres during a defined period of time are included in the survey. A defined intake period, which should not exceed 12 months, identical for all centres included in the survey, should result in a balanced sample, as centres are represented according to their burden of cases. This allows direct estimation of the prevalence of resistance from the proportion calculated in the sample. An unbiased estimate of the prevalence of drug resistance necessitates the inclusion of a specified minimum number of centres (i.e. 30) if there has to be a good probability of including centres of different types (clinics, dispensaries and hospitals of different sizes) scattered throughout the country.

Population-proportionate cluster sampling

To avoid the risk of drawing a sample that misses the largest diagnostic centres, a weighted cluster sampling technique can be used. Based on a list of all diagnostic centres with the numbers of newly-registered patients per year, a cumulative population list is compiled. Assuming that the minimum recommended number of 30 clusters is selected, the total number of patients registered per year in all the centres is divided by 30 to obtain the sampling interval.

The participants were provided with exercises for the sampling techniques, their problems discussed and sorted out.

4.3 Laboratory Techniques for Resistance Determination with Emphasis on Quality Assurance

Before the commencement of hands-on activities in the laboratory, the participants were briefed about the importance of quality, implementation of internal quality control measures, development of standard operating procedures, participation in external quality assessment schemes and integration of quality system in the culture of their respective laboratories to make them aware of the consequences of poor quality in any laboratory activity, especially the surveillance of drug-resistance in TB.

Presentation was also made by Dr Paramasivan on biosafety measures and shipment of infectious material that are essential in any laboratory that is undertaking work on *Mycobacterium tuberculosis*. The participants were taken around the newly-constructed BSL-III facility at the Institute to familiarize them with modern containment practices that are being considered as a prerequisite for handling drug resistance work on mycobacteria.

The participants were briefed about the availability of various techniques to determine the susceptibility of mycobacteria to antimicrobial agents. Drug susceptibility tests may be performed by either the **direct** or **indirect** method. For the **direct method**, the inoculum is a digested and decontaminated sputum (or other clinical specimen) in which AFB may be demonstrated in stained smears. In this method, the inoculum is truly representative of the bacillary population present in the specimen. The advantage of this method is that the drug susceptibility test results are available along with the culture results (by 3-4 weeks).

The **indirect test** is used for specimens that are smear-negative but culture-positive or when the growth in the control slope of the direct test is inadequate. Further, in the indirect test, the inoculum is standardized but at the same time is not truly representative and hence there is a chance of selecting a proportion of susceptible or resistant bacilli from the slope. For this reason, the inoculum is prepared by using a representative sweep of the entire surface of the growth on the slope.

The indirect sensitivity tests

There are three general methods used for determining drug susceptibility of mycobacteria by the indirect method: the absolute concentration method (MIC method), the resistance ratio (RR) method, and the proportion method. When properly standardized and performed, all three methods have been shown to be equally satisfactory.

The absolute concentration method uses a standardized inoculum grown on drug-free media and media containing graded concentrations of the drug(s) to be tested. Several concentrations of each drug are tested, and resistance is expressed in terms of the lowest concentration of the drug that inhibits growth; i.e., minimal inhibitory concentration (MIC). This method is greatly affected by the inoculum size and by the viability of the organisms.

The resistance ratio method compares the growth of unknown strains of tubercle bacilli with that of a standard laboratory strain ($H_{37}Rv$). Parallel sets of media, containing two-fold dilutions of the drug are inoculated with a standard inoculum prepared from both the unknown and standard strains of tubercle bacilli. Resistance is expressed as the ratio of MIC of the test strain to MIC for the standard strain in the same set. This test is also greatly affected by the inoculum size as well as the viability of the strains. In addition, any variation in the susceptibility of the standard strain also affects the resistance ratio of the test strain.

The proportion method enables a precise estimation of the proportion of mutants resistant to a given drug. Several 10-fold dilutions of inoculum are planted on to both control (drug-free) and drug-containing media; at least one dilution should yield isolated countable (50 -100) colonies. When these numbers are adjusted by multiplying by the dilution of inoculum used, the total number of viable colonies on the control medium, and the number of mutant colonies resistant to the drug concentrations tested may be estimated. The proportion of bacilli resistant to a given drug is then determined by expressing the resistant portion as a percentage of the total population tested. The proportion method is currently the **method of choice** and recommended by National Committee on Clinical Laboratory Standards (NCCLS), WHO and International Union Against Tuberculosis and Lung Diseases (IUATLD).

Various laboratory techniques for proportion method were first discussed in the lecture hall and subsequently demonstrated to the participants in the laboratory. These were:

➢ Pre-treatment of clinical material

➢ Preparation of Lowenstein Jensen (LJ) Medium

➢ Diluting various antimicrobial agents in recommended dilutions

➢ Inoculation onto LJ medium

➢ Reading the growth on LJ medium

➢ Calculating the ratio of resistant and sensitive strains

➢ Interpretation of results

Hands-on experience was also provided in important procedures and the problems experienced by participants discussed and resolved.

4.4 Mechanism for Networking and Sharing of Data with National Programme Managers

Dr Paramasivan described the importance of networking of laboratories and the structure and functions of existing network for drug resistance surveillance. Of the 22 supranational reference laboratories that exist in the world today, only one (Tuberculosis Research Centre, Chennai, India) is in the SEA Region. This supranational laboratory is linked with National Reference Laboratories for surveillance of drug resistance and also conducts external quality assessment for the national laboratories. It was suggested that the countries should have functional national reference laboratory which should forge strong linkages with the supranational reference laboratory as well as provincial/state laboratories. The use of modern tools of information technology should be accelerated to share data with national managers on a continuous basis to support the programme.

4.5 Country-specific Draft Plan of Action for Undertaking Surveillance of Drug Resistance in TB

Dr Rajesh Bhatia (WHO/SEARO) briefed the participants on the need for planning and the method of development of an action plan by identifying

various activities that are needed to achieve the specific objectives. Various parameters that need to be considered and included in the action plan are: description of the activity; time-frame, type of activity, person designated to undertake the same and the resources required to accomplish the activity, and supervision of the whole process. The participants developed country-specific draft action plans. Two of these were presented and discussed in the plenary session.

Various issues that need to be considered by the participants in the implementation of their respective plan of action were thoroughly discussed. Various technical and managerial problems raised by the participants were discussed and solutions suggested. Extensive discussions led to the formulation of recommendations that have been described in Section 5.

5.   RECOMMENDATIONS

To Member Countries

(1)   The knowledge and experience gained from the workshop in improving infrastructure and skills in the surveillance of drug resistance in TB should be utilized gainfully in supporting national efforts in TB control and strengthening of the DOTS programme.

(2)   All laboratories should strive to implement an efficient quality system so that the results generated are reliable and match with the needs of the users.

(3)   The Tuberculosis Research Centre, Chennai and the National Tuberculosis Institute, Bangalore, may be approached for any technical support and trouble-shooting in establishing surveillance of drug-resistance in TB.

To WHO

WHO should continue to provide technical support to all Member States in developing and strengthening infrastructure and skills in the surveillance of drug resistance in TB and should undertake independent appraisal of DRS in TB in the Region.

Annex 1

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Annex 2

PROGRAMME

Monday – 21 June 2004

0830 – 0900 hrs	Registration & Pre-test	
0900 – 0930 hrs	Inauguration	Dr P Kumar, Director, NTI Dr Rajesh Bhatia, Dr Hans Kluge, WHO
0930 – 0945 hrs	Scope of Workshop	Dr Rajesh Bhatia
0945 – 1015 hrs	Establishing a drug resistance surveillance system	Dr P Kumar
1030 – 1230 hrs	***Presentation of their country data***	*Participants (10 minutes for each country)*
1230 – 1330 hrs	Drug resistance surveillance – Current scenario in SEAR Countries	Dr C N Paramasivan
1330 – 1500 hrs	Enhancing quality of Laboratory services	Dr C N Paramasivan
1500 – 1545 hrs	*Lab Methods:* *Culture including pretreatment procedures*	Dr C N Paramasivan
1600 – 1630 hrs	Preparation of Lowenstein-Jensen based drug media (Theory)	Dr V H Balasangameshwara
1630 – 1730 hrs	Preparation of Lowenstein-Jensen based drug media (Practical demonstration in Lab)	Dr V H Balasangameshwara/ Dr C N Paramasivan and NTI Lab staff

Tuesday – 22 June 2004

0830 – 0930 hrs		Dr C N Paramasivan
0930 – 1030 hrs	Organization and survey outline	Dr V H Balasangameshwara
1045 – 1145 hrs	Sample size and sampling strategies	Mr K P Unnikrishnan
1145 – 1330 hrs	Exercises on Sample size and sampling strategies	Mr K P Unnikrishnan/ Dr V H Balasangameshwara/ Dr C N Paramasivan
1430 – 1530 hrs	***Drug susceptibility testing Methods***	Dr C N Paramasivan

| 1530 – 1730 hrs | Preparation of bacterial suspensions for proportion method: Practical demonstration | Dr V H Balasangameshwara/ Dr C N Paramasivan and NTI Lab staff |

Wednesday – 23 June 2004

0830 – 0900 hrs	Bio-safety Measures	Dr C N Paramasivan
0900 – 0930 hrs	Country presentation Bangladesh	BAN representative
0930 – 1100 hrs	EQA in smear microscopy:	Dr V H Balasangameshwara
1045 – 1145 hrs	Quality Control in culture and susceptibility testing of *M. tuberculosis*	Dr C N Paramasivan
1145 – 1330 hrs	Interpretation of results of proportion method: Theory	Dr V H Balasangameshwara
1430 – 1530 hrs	Interpretation of results of proportion method: Practical by participants	Dr V H Balasangameshwara/ Dr C N Paramasivan and NTI Lab staff
1545 – 1730 hrs	Drug Susceptibility testing methods for Non-tuberculosis mycobacteria	Dr C N Paramasivan

Thursday – 24 June 2004

0830 – 0900 hrs	Safe shipment of specimens and cultures	Dr C N Paramasivan
0900 – 0930 hrs	Interpretation of Survey data results	Dr V H Balasangameshwara
0930 – 1000 hrs	Components of country specific DRS plan	Dr C N Paramasivan
1000 – 1030 hrs	How to develop a country Plan of Action	Dr Rajesh Bhatia
1045 – 1145 hrs	Development of country work plans	Groups
1145 – 1330 hrs	Identification tests for *M. tuberculosis*: Theory	Dr C N Paramasivan
1430 – 1700 hrs	Identification tests for *M. tuberculosis*: Practical demonstration	Dr V H Balasangameshwara/ Dr C N Paramasivan/ NTI Lab staff
1700 – 1730 hrs	Identification of need for repeat practical work for Day 5	Dr V H Balasangameshwara/ Dr C N Paramasivan

Friday – 25 June 2004

0830 – 0900 hrs	Post test	
0900 – 1100 hrs	Suspension preparation and other lab work	Dr C N Paramasivan/ Dr V H Balasangameshwara
1100 – 1200 hrs	Presentation of country plan of actions	Dr Rajesh Bhatia
1200 – 1330 hrs	Panel discussion for trouble shooting	All faculty
1430 – 1630 hrs	Draft recommendations and valedictory	Director and All faculty