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Drug Resistance Surveillance in Tuberculosis

*Report of an Intercountry Training Workshop
Bangkok, 21–25 August 2000*

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1. INTRODUCTION

Tuberculosis continues to be one of the major public health problems facing mankind. As of now, about one-third of the world's population is infected with *M. tuberculosis* and there are about nine million new cases of tuberculosis and three million deaths occur worldwide annually. Developing countries contribute 95% of tuberculosis cases and 98% of TB deaths, of which 75% are in the economically productive age group. The burden has been increasing over the years among SEAR countries. Five of the 20 countries with a high burden of tuberculosis worldwide are from SEAR: Bangladesh, India, Indonesia, Myanmar and Thailand. Everyday, more than 1 500 people die in the Region of tuberculosis. Further the spread of HIV epidemic, increase in the level of drug-resistant tuberculosis, especially multidrug resistant TB poses a serious challenge to the epidemiology and control of TB in this Region.

Since laboratory diagnosis plays a pivotal role in the diagnosis and monitoring of tuberculosis patients, WHO SEARO has, over the years, been consistent in its efforts in providing technical support by way of training, developing standard laboratory manuals, SOPs, quality assurance programmes and networking of laboratories. As a part of these activities, a workshop on laboratory methods for TB control was successfully conducted at Jakarta, Indonesia, in April 1999, concentrating particularly on the key diagnostic component "sputum microscopy" and introduction of quality assurance in the networking of microscopy laboratories. This exercise has resulted in developing a National QC networking of "sputum microscopy" among Member Countries.

Although the preceding few years witnessed a remarkable progress in the diagnosis and phased implementation of DOTS in several of the SEAR countries, reliable data on the prevalence of drug resistance are not readily available. Even the available data from SEAR countries could not be taken with certainty due to limitations in the practice of standardized laboratory methods with the exception of countries such as India, Nepal and Thailand which participated in the global DRS. Besides, highly populous and diverse

countries like India and Indonesia require the participation of several regional laboratories to obtain realistic data for the respective countries. This workshop was therefore organized with the following objectives:

2. OBJECTIVES

- (1) To orient the participants about the sampling techniques required in undertaking community-based drug resistance surveillance in tuberculosis;
- (2) To impart skill on appropriate and uniform laboratory methodology for culture and drug susceptibility testing of *M. tuberculosis* and interpretation and reporting of the laboratory results;
- (3) To demonstrate techniques on QA in laboratory diagnosis of TB, with special reference to drug susceptibility testing methods, and
- (4) To develop country-specific plan of action on drug surveillance, including QA programme.

3. INAUGURAL SESSION

The opening ceremony was held at the Tuberculosis Division, Department of Centre for Disease Control, Ministry of Public Health, Bangkok, Thailand. Dr Nadda Sribhaya, former Director-General of Health Services, Thailand chaired the session. Dr Pasakorn Akarasewi, Director of TB Division welcomed the participants and other invitees. The objectives and mechanics of the workshop were explained by Dr Sudarshan Kumari, RA-BCT/WHO-SEARO. Mr Richard B. Kalina, Ag. WR-Thailand read out the message of the WHO Regional Director.

In his message, the Regional Director underlined the importance of collective action among the countries of the Region in the implementation of standardized methods in laboratory practices, early participation in the global DRS programme, and observance of strict internal quality control measures. He also recalled on this occasion, an initiative called " STOP TB' launched by the Director-General of the WHO, Dr Gro Harlem Brundtland at the World Lung Health Conference, Bangkok in November 1998 and called for the collective commitment to halt this scourge.

The workshop was conducted by the staff of WHO/SEARO and temporary advisers from India and Thailand. It was attended by 14 participants from Bangladesh, India, Indonesia, Myanmar, Sri Lanka and Thailand (See annex 1 for list of participants). Most of the participants were experienced laboratory persons actually involved in providing laboratory support to tuberculosis control programme in their respective countries. See Annex 2 for the programme followed in the workshop.

4. TECHNICAL SESSION

4.1 Introduction of Participants and Pre-test

Before start of the session, the participants were provided with a questionnaire, designed to obtain the participants' background, including their level of education and experience in laboratory practice, and to elicit their knowledge, attitude and practice (KAP).

A representative from each country made a brief presentation explaining the tuberculosis situation of their respective countries/regions and also the type of laboratory methods practiced. A few among them also presented available data on drug resistance.

4.2 Tuberculosis Incidence in South-East Asia

TB has been identified as a major killer disease accounting for more than 1 500 deaths daily in SEAR. With about 5% of global land area and 25 percent population, SEAR accounts for 42% of the global tuberculosis burden and one-third of global TB deaths. Further, the contribution of HIV epidemic to this threat and increase in the prevalence of MDR-TB with a poor cure and higher mortality rate is a significant threat to TB control programmes. Although SEAR has made some pioneering contribution towards TB control such as methodology for conducting national sample survey; efficacy and feasibility of case-detection by sputum microscopy; successful outcome of home-based treatment; efficacy of intermittent chemotherapy and discovery of the principles of DOTS. Despite these contributions, there is continuous burden of TB in SEAR due to lack of political will and poor allocation of funds, inadequate and irregular supply of anti-TB drugs, poor quality of drugs, and

lack of supervision. Since the control of TB by DOTS has proved effective in a big way, the importance of strengthening the key diagnostic methods such as sputum microscopy and other bacteriological diagnostic methods including determination of drug resistance profile was stressed. Since the level of initial drug resistance serves as an epidemiological marker of bacterial transmission in the community, it is important to follow internationally accepted methodology for setting up drug susceptibility testing including quality assurance to avoid wide variation in drug resistance as seen at present in Member Countries.

5. SUMMARY OF COUNTRY PRESENTATIONS

It was understood from the country presentations that a certain level of quality assurance is being practised in sputum microscopy. However, networking of laboratories at various levels, periodic analysis and corrective measures are not being practised regularly at present. Their presentation brought forth the importance to be given to this basic technique and the need to fulfill this obligation by all concerned at the earliest possible time in their respective countries. Further, it was learnt that different techniques are being practised at present for homogenizing and decontaminating sputum samples, types of culture media used for the growth of tubercle bacilli, techniques employed for interpreting results and definition of drug resistance, differentiation of *M.tuberculosis* from Non-Tuberculous Mycobacteria (NTM) and standardized source of drugs for setting up drug-susceptibility testing. Except for three countries, namely India, Nepal and Thailand, other countries of the Region are yet to participate in the Global Drug Resistance Surveillance sponsored by WHO. The importance of linking NGOs, universities, national reference laboratories, wherever possible, was also discussed. The answers to questionnaires and presentations made by the participants provided salient information which was utilized for reorganizing various laboratory methods and corresponding presentations on various techniques.

6. LABORATORY TECHNIQUES

All the practicals were conducted in an interactive manner with corresponding laboratory sessions. Hands-on training was provided to individual participants on all standard conventional laboratory diagnostic

methods of tuberculosis. Special efforts were also made to teach safe laboratory practices, specifically to contain the spread of dangerous aerosols while manipulating biological samples for culture and drug-susceptibility testing. Participants were also trained about planning laboratory requirements for setting up bacteriological methods in the diagnosis of tuberculosis.

6.1 Specimen Collection, Transport and Processing

The value of overnight and early morning collections and spot collection of sputum samples for smear and culture and their related merits in smear and culture positivity was described. Practical demonstrations were made to participants in storing and transport of sputum in specific containers with inexpensive preservative such as 1% cetylpyridium chloride plus 2% sodium chloride solution (CPC) to retain viability of tubercle bacilli upto 7–10 days. Homogenizing and decontaminating sputum samples by Petroff's method was also demonstrated. They were also taught the importance and critical nature of exposure time to alkali, direct processing of CPC containing sputum samples without further pre-treatment procedure and the temperature build-up in the specimen during centrifugation.

6.2 Culture, Identification and Drug-Susceptibility Methods

The participants were given hands-on training on the preparation of egg-based Lowenstein-Jensen (LJ) medium. Various precautions to be followed in the preparation of drug-free and drug-containing LJ medium were demonstrated. Preparation of critical concentration of various primary anti-TB drugs, such as, isoniazid, streptomycin, rifampicin, ethambutol and appropriate solvents to be used wherever necessary were explained. Great deal of attention was paid in the preparation of bacterial inoculum for drug-susceptibility testing and in the calculations of drug-susceptibility results. The participants were made to practise these two exercises several times and their results were compared with the known standards. They were also made to set up drug-susceptibility testing by the standard procedure, proportion sensitivity testing (PST) for primary anti-TB drugs. They were also trained to take reading on *M.tuberculosis* cultures which had been already set up for sensitivity and carry out calculations to express the results obtained. Quite a few simulation experiments were performed for the participants for interpreting drug-sensitivity readings. Results obtained by the participants were compared and discussed.

The colony morphology of several mycobacterial species by visual demonstration of their appearance, several key tests, such as niacin production, nitrate reduction, stability of catalase at 68°C and presence or absence of growth in LJ medium containing para-nitro benzoic acid were demonstrated. Participants also performed these tests independently in groups.

6.3 Drug-resistance Surveillance

The methodology of conducting drug-resistance surveillance adhering to the WHO guidelines was described in great detail. Different sampling strategies, various steps involved in the conduction of DRS, sample size, the importance of eliciting history of previous treatment from patients, collection and transport of sputum samples, filling up of sputum collection and reporting forms, analysis and interpretation of data were demonstrated in great detail. The actual DRS data obtained as a part of global DRS programme in India and Thailand was also described with suitable illustrations, including three rounds of EQAS studies completed recently in India. The participants were encouraged to plan strategies for conducting DRS in their respective countries. The basic skeletal proposal thus developed by the individual DRS were also discussed. While doing this, certain key data capture forms that require to be employed in global DRS and the model package Epi-Info (WHO, Geneva SBR TB Version 2.0) were introduced for their use in analysis of DRS data.

6.4 Recent Diagnostic Methods for Culture, Identification and Drug-Susceptibility Testing

For updating knowledge on the recent laboratory methods, participants were oriented to methods, such as BACTEC 460 TB, MGIT, Septi-Check, E-Test and Alamar Blue Assay etc. In addition, molecular-based diagnostic methods for identification and drug resistance detection and the relative merits and limitations in adopting such techniques for SEAR countries were explained. The participants' views on the above subject highlighting their misuse, sub-optimal usage and over-dependence on these methods were discussed.

7. QUALITY ASSURANCE MEASURES

Various Internal Quality Control (IQC) measures to check the optimal growth of tubercle bacilli on Lowenstein-Jensen medium, correct preparation of LJ media, LJ media containing various anti-TB drugs for drug-susceptibility, homogenization and decontamination procedures, inoculation and incubation procedures, culture examination, enumeration of number of colonies and their identification as well as reading and reporting of results were discussed. In addition, IQC measures for inoculum size, range of drug-concentration, source of drugs, incubation temperature, maintenance of controls for various procedures, including identification methods were discussed with suitable practical aids. The periodicity with which the IQC measures are to be organized for various methods in different laboratory settings were also discussed.

The principles and practice of External Quality Assessment Scheme (EQAS) was further described with illustrations of EQAS undertaken by one of the SEAR countries with a laboratory at Brisbane, Australia. The networking of laboratories organized by WHO and the importance of the national laboratory's participation in networking were dealt in details. The participants were encouraged to draw out action plans to illustrate IQC measures and EQAS for DRS in their respective countries. These presentations made by the participants reflected their eagerness to practise standard laboratory methods and also enhanced their perception about quality assurance.

8. EVALUATION OF THE WORKSHOP

A separate session was organized to obtain the participants' perception about the course contents and facilitators' performance. They were also encouraged to offer anonymous suggestions for improvement. Detailed analysis had shown that participants were by and large satisfied about the course contents, facilitators' performance, practical sessions and reference materials provided. However, some of the participants suggested video tape demonstrations and distribution of lecture materials prior to the teaching sessions.

9. PROBLEM-SOLVING AND CLOSING SESSION

In this lively session, laboratory exercise results, pre-and post test calculations of PST and various quality assurance measures were discussed. Participants were also provided details about reliable sources from where reagents, chemicals, standard strains and drugs could be procured. The maintenance of control strains, media, reagents and drugs and methods to check their quality were explained and necessary hand-outs and control strains distributed. This workshop has helped senior laboratory personnel to practise uniform laboratory methods for culture and drug-susceptibility for the first time. It also gave them an opportunity to update their knowledge, optimize the existing facilities within their respective systems and develop methods for IQC and future plans to participate in EQAS. Norms were established for quality assurance and networking of laboratories at the national level, among SEAR national laboratories and with WHO collaborating centres in the Region.

10. RECOMMENDATIONS

- (1) The participants should initiate drug resistance surveillance adhering to the global DRS methodology as learnt in the workshop in coordination with their respective TB programme managers.
- (2) All participating laboratories should adhere to the practices of internal quality control using SOPs provided during the workshop.
- (3) WHO should explore the possibility of identifying an appropriate laboratory within the Region for starting an External Quality Assessment Scheme in drug susceptibility testing of *M.tuberculosis*.
- (4) To assess the progress made by the participating laboratories with respect to their laboratory services, WHO should design a questionnaire and send it to them periodically. The participants should complete the same and return to WHO for evaluation.
- (5) For better inter-laboratory communication and referral, the participants should develop liaison with collaborating centre/national centre for TB diagnosis situated in the country/Region.
- (6) The participants should arrange similar workshops in their respective countries to disseminate correct information on laboratory practices.

Annex 1

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Annex 2

PROGRAMME

Monday, 21 August 2000

- 09.00 – 09.30 Registration
- 09.30 – 10.00 Opening ceremony
Welcome address
RD's address
Objectives and mechanics of the meeting
Special addresses – other dignitaries
- 10.30 – 11.00 Introduction of participants and Pre-Test
- 11.00 – 12.30 Introductory lectures
Drug Resistance Scenario in SEAR
Dr Sudarshan Kumari
Principles and Practice of Quality Assurance
Dr. Rattan Ichhpujani
- Need for Drug Susceptibility Testing*
 - Description of different testing methods*
 - Advantages and Disadvantages*
 - Choice of methods*
- **Dr. C.N. Paramasivan*
- 13.30 – 14.30 Proportion Sensitivity Testing (PST) (Lecture)
(Methodology)
Dr. C.N. Paramasivan
- 15.00 – 15.30 Sampling techniques for community based drug resistance surveillance
Dr. Rattan Ichhpujani
- 15.30 – 16.30 Reading and interpretation of PST (Lecture)
Dr. C.N. Paramasivan

Tuesday, 22 August 2000

- 08.30 – 09.00 BACTEC/Other recent methods (Lecture)
Dr. Urvashi Singh
- 09.00 – 10.00 Drug media preparation (Lecture)
Dr. Urvashi Singh

- 10.30 – 12.30 Demonstration – Media preparation (Practical)
Mr. Somsak Rienthong / Mrs. Dhanida Rienthong
- 13.30 – 14.15 Principles and Practice of DOTS
Dr. Rattan Ichhpujani
- 14.15 – 16.30 Demonstration – Setting up of Drug Susceptibility Testing (DST)
(Practical)

Wednesday, 23 August 2000

- 08.30 – 10.00 Basic identification tests (Lecture)
Dr. Urvashi Singh
- (PNB, Niacin, Nitratase, Stability of catalase at 68°C)
- 10.30 – 12.30 Demonstration, Reading and Interpretation (Practicals)
Mr. Somsak Rienthong / Mrs. Dhanida Rienthong
- 13.30 – 16.30 Demonstration – Identification test (Practical – contd.)
Mr. Somsak Rienthong / Mrs. Dhanida Rienthong

Thursday, 24 August 2000

- 08.30 – 10.00 Quality Assurance in DST (Lecture)
Dr. C.N. Paramasivan
- 10.30 – 12.30 Hands on training – DST setting up (Practical)
Dr. C.N. Paramasivan, Mr. Somsak Rienthong / Mrs. Dhanida Rienthong
- 13.30 – 16.30 Hands on training – Reading and Interpretation (Practical)
Dr. C.N. Paramasivan, Mr. Somsak Rienthong / Mrs. Dhanida Rienthong

Friday, 25 August 2000

- 08.30 – 09.00 Group work – Test on PST Calculations
Dr. C.N. Paramasivan, Mr. Somsak Rienthong / Mrs. Dhanida Rienthong
- 09.00 – 10.00 Development of country specific plan of action
Group Work
- 10.30 – 11.30 Development of country specific plan of action (contd.)
Group Work
- 11.30 – 12.00 Post Test
- 12.00 – 12.30 *Discussion and recommendations*
Dr. Sudarshan Kumari

