REPORT


WORLD HEALTH ORGANIZATION

Introduction

Professor W.C.S. Smith welcomed all participants and stressed the importance of this workshop in getting national programmes and reference laboratories to come together to collaborate in setting up a sentinel surveillance system to monitor drug resistance in leprosy. The Vice-Minister of Health in Viet Nam, Dr. Nguyen Thi Xuyen, in her opening address welcomed the participants and thanked WHO for giving the opportunity to host this important workshop in Hanoi. She pointed out that this workshop is an important step towards improving the quality of the leprosy control services and it is very encouraging to see that all partners namely, the national programmes, research institutes and their laboratories and WHO are joining hands to set-up this surveillance system. The WHO Representative to Viet Nam Dr. Jean-Marc Olivé addressed the participants and read out the welcome speech from the Regional Director of the Western Pacific Region.

The participants selected Professor W.C.S. Smith as chairperson, Dr. Tran Hau Khang as co-chairperson and Dr. Paul Saunderson as rapporteur of the workshop. Agenda and list of participants are provided in annexes 1 and 2.

Setting the Stage

THE NEED TO MONITOR DRUG RESISTANCE

Professor Smith reviewed the history of leprosy chemotherapy and the achievements made during the period of the strategy of Elimination of Leprosy as a Public Health Problem. Looking back at the history of dapsone therapy it was pointed out that as it was used as a monotherapy treatment initially starting with low dosage, secondary dapsone resistance appeared during the mid-sixties and during the seventies primary dapsone resistance was reported from several field programmes. This lead to WHO introducing multi-drug therapy (MDT) using three drugs namely: rifampicin, clofazimine and dapsone. The elimination strategy played an important part in introducing MDT on a wide scale globally free of charge.
It also strengthened integration of leprosy services into the general health care system and improved service coverage in all endemic countries. It introduced simplified diagnosis and classification, increased political commitment and mobilised additional resources for leprosy. In order to maintain the above-mentioned achievements the current Global Strategy 2006–2010 has focused its attention towards sustainability and improving the quality of leprosy control services.

It was noted that among the various potential threats that could hinder on-going efforts to further reduce the burden of disease in endemic countries, the development and transmission of rifampicin-resistant leprosy is potentially a serious threat for future leprosy control programmes. In the past there was a high degree of complacency about drug resistance, mainly because the method available to detect drug resistance (mouse footpad inoculation) was difficult to perform, complicated, time consuming and expensive. This has resulted in a lack of information on drug resistance which, of course, is not evidence of absence of drug resistance. It was assumed that a combination of three drugs, if taken regularly would prevent the emergence of drug resistance and because reported treatment completion rates have been relatively high, it was generally regarded that the self-administered part of the MDT regimen was taken by the patient as prescribed. There is limited information on patient adherence with the unsupervised components of MDT. With the recent development of DNA sequencing methods to detect drug resistance, several reports of rifampicin, dapsone and ofloxacin resistance have been published and this has highlighted the importance of this condition and the need to monitor this systematically.

In addition, although other drugs are available as second-line treatment for drug resistance, their use in the field is perhaps not as simple as MDT and their efficacy and safety needs to be monitored as more patients are put on such second-line treatment. It also highlighted the need to develop better alternative regimens. The problem of drug resistance may or may not be acute at present, therefore, it is important that we collect data more systematically and monitor the trend carefully so that effective measures to combat this problem can be developed in future.

The objectives of the workshop were outlined as follows:

- To standardise manuals of procedures for collection, recording, reporting and transportation of samples from each participating centre so as to ensure quality control.
- Standardise methods and procedures for molecular testing of dapsone and rifampicin resistance.
- Standardise procedures for data collection, collation, analysis and reporting of results periodically.
- Agreement between national programmes, referral institutes and reference laboratories on the procedures for quality control.
- To strengthen networking and transfer of technology between national programmes and research institutes.

GLOBAL LEPROSY SITUATION

Dr. Myo Thet Htoon presented the current global leprosy situation, as reported to WHO by 126 countries at the beginning of 2008. He pointed out that globally, new case detection (NCD) has decreased significantly over the past 5 years, but in the last 2 years the decline has become less steep especially in the South East Asia Region which represents 74% of new
cases globally. India detected over 137,000 new cases in 2007 compared to over 139,000 in 2006 which shows a decline of only 1%. Among the top 18 countries (representing 94% of total global new cases) that are reporting more than 1000 new cases during 2007, 10 countries are showing an increase in new case detection compared to 2006. It is to be noted that although new case detection is declining globally, the trend at country level especially in the top 18 countries is not uniform. Reported treatment completion rates are also high; however, out of the 126 countries that submitted leprosy statistics only 16 submitted figures for treatment completion rates, which shows that further efforts are needed at country level to collect this important information. With regard to information on the number of relapsed cases, during the last 4 years approximately 40 countries have been reporting this data with a rather stable total of over 2400 cases per year. In absolute terms, Brazil, Ethiopia and China reported the highest numbers of relapsed cases, but there are questions about the degree of over or under-diagnosis. This is mainly due to the current definition of relapse, which is based mainly on clinical signs, and the inherent difficulties encountered in the field in differentiating relapse from reactions.

RECENT REPORTS ON DRUG RESISTANCE

Dr. M. Matsuoka gave a comprehensive historical overview of drug resistance in leprosy. In 1964, dapsone resistance was first confirmed in the mouse footpad (MFP). Clofazimine resistance isolates were reported in three cases from 1982 to 1996, but it has not been reproduced and so can be regarded as negligible. There are no known cases of resistance to minocycline or clarithromycin in *M. leprae*. Resistance of *M. leprae* to dapsone, rifampicin and quinolones can be associated with various genetic mutations in the *folP1*, *rpoB* and *gyrA* gene respectively, allowing detection of drug resistance to key components of multi-drug therapy (MDT) by molecular methods with a high degree of accuracy.

Reports of rifampicin, dapsone and quinolone have been published by several scientists (Cambau, Cole, Honore, Kai, Lee, Lopez-Roa, Matsuoka, Ozarmagan, Williams and You) in their respective laboratories using molecular biology techniques. Almost all dapsone resistant isolates with low degree in MFP test do not reveal mutation in the *folP1* gene but it is of no concern clinically since the mouse dose of 0.0001 gm per 100 gm of diet is far lower than the dose prescribed for a patient. In a recent study carried out among relapsed cases by Matsuoka et al., rifampicin resistance was reported from Myanmar (2/24 samples) and Indonesia (2/10 samples). However, none was found in 19 samples collected from the Philippines. Dapsone resistance was reported in 2/24 samples from Myanmar, 1/10 samples from Indonesia and 5/19 samples from the Philippines. Among new or recent cases, rifampicin resistance was reported in 4/121 cases in Indonesia and 1/54 cases in Myanmar. Dapsone resistance was reported in 1/121 cases in Indonesia, 4/54 cases in Myanmar, and 2/77 in the Philippines.

Amino acid substitutions at the following locations have been observed in the isolates resistant to the following anti-leprosy drugs in *M. leprae*.

- Dapsone at *folP1*: threonine (AAC) at 53 and proline (CCC) at 55
- Rifampicin at *rpoB*: glycine (CAG) at 407, aspartic acid (GAT) at 410, histidine (CAC) at 420, serine (TCG) at 425 and leucine (CTG) at 427
- Quinolone at *gyrA*: glycine (GGC) at 89, alanine (GCA) at 91, although mutations for serine (TCG) at 92 and aspartic acid (GAC) at 95 have not been detected, these mutations probably confer quinolone resistance according to the findings in *Mycobacterium tuberculosis*. 
RECENT ADVANCES IN DNA SEQUENCING: MOLECULAR METHODS FOR
DETECTION OF DRUG RESISTANCE

Professor Emmanuelle Cambau presented further information about molecular methods. The
basic concept is that the genotype (mapped by molecular methods) predicts the phenotype,
which is measured by the mouse footpad (MFP) method. Various studies have shown the high
concordance between the MFP results and molecular methods, the main exception relates to
low-level resistance to dapsone, not detectable by molecular methods, which is thought to be
clinically insignificant. One study showed 97% concordance for rifampicin resistance with
MFP method. A major disadvantage with the MFP method is the lack of growth of the bacilli
in a significant proportion of cases, not to mention the practical difficulties of the technique.

Resistance to all current anti-leprosy drugs (except clofazimine) can be linked to specific
gene mutations. The molecular methodology involves amplification of the DNA from the
specimen by PCR, followed by DNA sequencing and checking for mutations against a known
database. Although DNA sequencing is the standard method, if available, various simpler
methods are being developed. Professor Cambau is developing a DNA strip method that
might be used in the future instead of DNA sequencing. Professor Cambau suggested that the
numbering system for gene sequencing needs to be agreed upon (using either the \textit{M. leprae} or
\textit{E. coli} numbering systems) as well as a common database of relevant mutations.

Discussion centred on various practical matters, including the type of specimen which
should be used (biopsy or skin smear). Further discussion favoured the less invasive
procedure of skin smear as the norm; although a biopsy could be done if possible in addition
to skin smear, should this be acceptable to the patient. It was suggested that the
bacteriological index (BI) needed to guarantee a definite result was around 3+. However, it
was suggested that any relapse should be tested, even if the BI is below 3+, as a valid reading
may still be possible. For the purpose of starting a sentinel surveillance network for drug
resistance which will be initially looking at MB relapsed cases as a potential risk group, the
experts have suggested including MB relapsed cases with a BI of at least +2. This would give
a high probability of successfully extracting DNA from skin smear samples and completing a
successful DNA sequencing result. The possibility of mixed populations of bacilli was
mentioned, with the suggestion that the MFP method may cope better with this problem than
the molecular methods, but this is not known for certain.

As the technology for extracting DNA from skin smear samples improves a review is to
be made regarding the patient inclusion criteria for drug resistance surveillance, for example
other risk groups such as defaulters and new cases.

COUNTRY PRESENTATIONS: CURRENT PRACTICES FOR DIAGNOSIS, REFERRAL
AND MANAGEMENT OF RELAPSES

Brazil

Dr. Samira Bührer gave a presentation on current practices regarding diagnosis, referral and
management of relapses in Brazil. Guidelines on the diagnosis and management of relapses
have been available since 1998, and regular meetings are held to keep staff aware of them.
The criteria for MB relapse are based on the appearance of new lesions or the exacerbation of
old ones along with newly-affected nerves supported by a positive BI and active MB pattern
histopathological report. All reported relapses are supposed to be checked at state level, but
this may not always be applied in the field. In a recent study of 142 suspected cases of relapse,
only 104 were confirmed, which included cases treated with dapsone monotherapy also. The proportion of all cases that are designated as relapses rose from 2.5% in 2001 to 3.6% in 2007, but the validity of these figures is questionable.

A case–control study of relapse is to be started in five states involving seven centres. The study will be applying clinical, histopathological and molecular methods, MFP studies and a serological test (PGL-1) to confirm relapse and identify drug resistance.

**Ethiopia**

Dr. Elizabeth Kassa presented the situation in Ethiopia. A stable number of 4000 to 5000 new cases are being detected each year and approximately 7% are children; 12% have grade 2 disabilities and 80–90% are classified as MB. There is only one specialised hospital for leprosy in Ethiopia, namely, ALERT, founded in 1965, which currently diagnoses about 400 new cases per year. In addition, 1200 leprosy cases with complications are seen per year and about 500 of them are cases referred from the regions for the management of complications.

The Armauer Hansen Research Institute (AHRI) was established in the same compound in 1966 for research in leprosy. It has contributed a lot to the expansion of our knowledge of leprosy.

Countrywide, around 200 to 300 MB relapses and four to 26 PB relapses are identified each year. In Ethiopia, PB cases that develop MB leprosy after completing PB-MDT are not classified as a relapsed case but categorised as a misclassified case.

There was discussion about referring to relapsed cases in relation to (or even as a proportion of) new cases detected in any particular year. Clearly there is no direct relationship between relapsed cases and current new cases, but it may be helpful to indicate the breakdown (i.e. new case, relapsed, return from default, etc.) of all cases starting treatment each year, to show drug requirements and as a general indication of the situation.

**India**

Dr. P.L. Joshi shared the current situation in India regarding relapses. The national guideline has instructed that relapsed cases are to be suspected in the peripheral clinics and referred to secondary and tertiary levels for confirmation. Once confirmed, relapsed cases are to be put on MDT as per WHO operational guidelines. As of August 2008, 277 cases were suspected to be relapsed cases at PHC level, out of which 182 have been confirmed at district hospitals as a case of relapse. Dr. Joshi stated that laboratory services are being utilised at secondary and tertiary level institutions for confirmation of relapses. A reporting system has been developed in the country for monthly routine reporting of suspected and confirmed relapsed cases. On the issue of priorities, Dr. Joshi stated that surveillance of drug resistance and establishing the need for effective and safe new drug regimens is a priority today.

The experience of the 18 Leprosy Mission Hospitals in India, with a total of 1089 beds available for providing care to people affected by leprosy was presented by Dr. Rajan Babu. The hospitals in total are detecting around 5000 new cases a year and admit around 10 000 patients for treatment of complications and reconstructive surgery. The Stanley Browne Laboratory at New Delhi is the main laboratory supporting these hospitals in providing facilities for various histopathological and immunological tests. The criteria used for diagnosing a case of relapse is based on WHO Operational Guidelines. In 2007, three MB relapses after treatment were diagnosed in the above-mentioned centres. During the period January to June 2008, three cases were diagnosed with relapse.
Dr. Mannam Ebenezer shared experiences from the Schieffelin Institute of Health Research and Leprosy Centre in Karigiri, Tamil Nadu. The institute detects around 250 new cases each year with 80% of them MB cases. From 2004 to 2007 drug resistance studies for dapsone and rifampicin were carried out using MFP and molecular studies. Out of 107 untreated new MB patients with a BI of more than +2 none showed resistance to either dapsone or rifampicin using molecular methods. However, five showed dapsone resistance at high concentrations. Among the group of 22 new cases (that were never treated with MDT and were put on MDT at the start of treatment but later did not show one log decline in BI after 12 months of MDT) no drug resistance was found either with MFP or molecular methods. However, among seven cases of suspected MB relapse one patient showed dapsone resistance by both molecular methods and MFP (at high concentration). There was some loss of concordance between the MFP (which also showed dapsone resistance in five of the new cases – primary resistance) and molecular methods, but there may have been weaknesses in the protocols for administering dapsone to the mice.

Myanmar

Dr. Kyaw Kyaw presented the situation in Myanmar. It was pointed out that Myanmar in the past had a very high number of cases and the cumulative number of patients that have been treated with MDT was about 270,000 cases. This meant that the potential for detecting relapses is very high and in addition, around 1000 new cases were treated with a combination therapy of rifampicin, ofloxacin and minocycline (ROM) as part of a WHO clinical trial during the mid-1990s. There are two referral hospitals for leprosy, one near Mandalay and one in Mawlamyaing, and two special skin clinics which are part of the tertiary care hospitals in Yangon and Mandalay. The above-mentioned four centres are regarded as referral centres supporting the integrated leprosy control services operating in each township.

In 2007, a total of 22 relapsed cases were reported. At the Central Special Skin Clinic in Yangon General Hospital, 106 relapsed cases were diagnosed during the period of 1990 and 2007, including one multi-drug resistant case. Two early relapses (after 1 and 8 years, respectively) have been noted in a cohort of 200 MB cases treated with 1-year MB-MDT, but no drug resistance mutation was identified in the PCR study. Suspected relapsed cases are identified in the periphery, reviewed and confirmed at regional level and in some cases where the diagnosis of relapse was still uncertain, cases were then referred to the referral centres in Yangon and Mandalay for confirmation. The criteria for relapse are based on finding new skin lesions and new nerve involvement and/or an extension of previous lesions with signs of activity. An increase in BI is regarded as the key indication of the multiplication of \textit{M. leprae}, and the Morphological Index (MI) is also considered a useful additional tool in the diagnosis of relapse. An important issue generally faced in the programme was the lack of clinical experience among clinicians as well as specialised leprosy workers in the peripheral units, so that reaction cases may be misdiagnosed as relapses and treated with MDT.

Vietnam

The leprosy situation in Vietnam was presented by Dr. Tran Hau Khang from Vietnam. It was highlighted that the new case detection in Vietnam has declined quite steeply since 1997 from 3.65 to 0.66 per 100,000 in 2007. During 2007, 552 new cases were detected with a MB proportion of 68% and child proportion of 4.5%. The MB proportion has increased to between
60% and 70% in recent years. The grade 2 disabilities proportion remains high at 16% to 18%. Interestingly, the actual number of cases with grade 2 disabilities was constant from 1983 to 1998 at around 600 per year, but it has decreased since then and has halved in the last 5 years, from over 200 in 2002 to 102 in 2007, although the proportion has remained the same because the number of new cases is also declining.

Relapses range from two to 27 cases per year over the last 9 years. During 2007, eight relapsed cases were reported; all of them were MB cases. As of the end of June 2008, six MB relapsed cases have been reported. In addition, there have been 13 relapses following chemotherapy trials on various regimens of ofloxacin.

Currently, relapsed cases are being confirmed at regional dermatological hospitals and by the National Institute of Dermatology and Venerology (NIDV) at Hanoi.

LABORATORY ASPECTS ON SURVEILLANCE FOR DRUG RESISTANCE

Ethiopia

Dr. Demissew Beyene presented the functions and activities of the Armauer Hansen Research Institute (AHRI) in Addis Ababa, Ethiopia. AHRI is a well-established research centre performing research mainly on mycobacterial diseases and currently it is administratively linked with the ALERT hospital, and has its own Scientific Advisory Board (SAB). AHRI has a functional network within ALERT campus and with different universities in Ethiopia, the National Tuberculosis and Leprosy Team (NTLT), Non-Government Organisations (NGOs) and regional health activities. It has consortium, Collaborative and student projects. These projects involve different study sites throughout the country studying TB, leprosy, leishmaniasis, malaria and meningitis, and make a significant contribution to training at various levels, including PhD candidates. There is no current study of drug resistance in leprosy, but the capacity (both in terms of human resources and physical infrastructure) to be involved in the proposed surveillance project is present. Specifically, AHRI scientists have plenty of experience with PCR and equipment is available. AHRI has the experience of receiving samples as to the Standard Operation Procedure (SOP) from different study sites and also sending samples overseas for quality control purposes as well as when there is a gap in technology. Availability of data management unit for data entry, data cleaning and data analysis is an addition. Therefore, the experience and capacity of AHRI justifies its potential to be a partner for the global sentinel surveillance of drug resistance in leprosy being a referral laboratory within Ethiopia.

The possibility of training a scientist in Dr. Matsuoka’s laboratory was discussed and it was agreed that this could be done as long as AHRI can find funds for this scientist’s travel and stay in Tokyo. However, for this current sentinel surveillance to start moving forward in Ethiopia it is important that samples be sent to Professor Emmanuelle Cambau’s laboratory in Paris for DNA sequencing while the laboratory in AHRI gains more experience in the technique. As the representative from the national programme was unable to attend this meeting, WHO will approach the national authorities to see if they will participate in the surveillance network. The interim arrangements of getting skin smear samples tested in Professor Emmanuelle Cambau laboratory will be explained. This can continue until AHRI can perform the tests in Addis Ababa.

AHRI will also help in linking up with other referral hospitals in Ethiopia that can be recruited as additional national sentinel sites in the future. At present ALERT will be the
proposed sentinel site where relapsed cases will be diagnosed and managed along with systematic collection of skin smear samples for molecular testing.

India

Dr. Rupendra Jadhav explained about the setup of the Stanley Browne Laboratory (SBL) which is based at the TLM Community Hospital in Shahdara, Delhi. The laboratory supports a large network of hospitals in endemic areas with PCR capabilities and excellent collaborative links within India and abroad. SBL has also been involved in different research projects where protocols for clinical sample collection from field areas, storage and transport were developed and executed. SBL will be coordinating sample collection, processing samples for DNA extraction and carrying out PCR amplification. For the DNA sequencing part of the process, it will be sub-contracting this work either to a national facility in New Delhi (at a reasonable cost with a turnaround time of about 4 days) or it will link up with the Central JALMA Institute for Leprosy and Other Mycobacterial Diseases in Agra, India.

DNA sequencing for other research purposes has been out-sourced to private laboratories in developed countries routinely as it has been found to be cost-effective. Similar innovative approaches could be tried out under the current surveillance system in India as the technology for DNA sequencing is readily available in many government institutes and private laboratories. It was noted that quality control could also be easily built into the project design.

The Central JALMA Institute for Leprosy and Other Mycobacterial Diseases is also another reference laboratory which has agreed in principle to participate in the surveillance system. Studies on drug resistance are currently being carried out at this institute using DNA sequencing techniques. Along with the laboratory at the Schieffelin Institute of Health Research and Leprosy Centre in Karigiri, Tamil Nadu, the laboratories will be co-ordinating the testing of samples for drug resistance. The laboratory at the Schieffelin Institute of Health Research and Leprosy Centre will also be out-sourcing DNA sequencing tests.

Japan

Dr. Matsuoka described the procedures used in his laboratory in Tokyo for DNA sequencing based on samples collected by standard slit skin smears as outlined in the surveillance guidelines. The importance of using a stainless steel blade in taking slit skin smears to prevent rust was emphasised, as was preserving the samples in 1 ml of 70% ethanol. There is no need to keep samples in a refrigerator and it is regarded as non-infectious for shipping purposes as it is preserved in 70% ethanol solution. A possible alternative is the use of FTA card (similar to filter paper) which has been known to preserve DNA very well. The usefulness of FTA card for this surveillance will be tested in the field settings in few endemic countries.

Steps in the protocol for extracting DNA were presented based on the agreed protocols outlined in the research projects carried out under other research initiatives. Procedures for direct PCR sequencing using Big Dye 1·1 Terminator Ready Reaction Mix were outlined. Sequencing results were shown and a case of silent mutation was illustrated.

Brazil

Dr. Philip Suffys presented the work done at the Oswaldo Cruz Foundation’s Laboratory of Molecular Biology applied to Mycobacteria in Fiocruz, Rio de Janeiro, Brazil. Recent work on
strain typing has been carried out at Fiocruz based on three basic methodologies. There are phylogenetic markers indicated by a number of SNPs. Strain typing by VNTR has been studied in various centres and has performed well in studies in Brazil. It was noted that it is important that all groups use the same reagents, which vary greatly in price, to get reliable results. The third method of distinguishing strains of *M. leprae* is through the study of drug resistance.

Currently work is being carried out on a series of 196 samples and so far two mutations have been found, neither of which was associated with resistance. Some paired samples (initial infection and relapse) have indicated the likelihood of re-infection in several cases, indicated by mutations in the *gyr*-A gene.

Experts at the meeting in Agra, India in November 2006 came to a consensus that the use of DNA sequencing technology and identifying mutations at specific locations will be the method of choice for predicting drug resistance. Based on this recommendation the participants agreed that for the surveillance of drug resistance DNA sequencing will be the only test used. However, should individual participating reference laboratories wish to carry out MFP studies in addition to DNA sequencing it is to be permitted.

**DISCUSSION ON THE GUIDELINES FOR GLOBAL SURVEILLANCE OF DRUG RESISTANCE IN LEPROSY**

*Introduction to the objectives and outline of sentinel surveillance*

Professor Smith initiated discussion on the Guidelines, which was currently in draft form. The main components of the surveillance system were explained. The overall aim is to establish a network (based on the participants of this workshop) and monitor trends in secondary drug resistance (rifampicin, dapsone and ofloxacin).

Issues relating to improved reporting of relapses cases at national level and selection of sentinel sites, need to standardise case ascertainment and definitions, operational issues (patient consent, sample collection, storage and transport) and the establishment of a global network focusing especially on the linkages between the referral centres and the respective reference laboratories were presented.

The sentinel sites should be in the key leprosy endemic countries and should have clearly defined catchments areas that were representative of the country. The areas need to have treated a reasonably large number of cases in the previous decade to ensure a minimum number of relapsed cases for surveillance. In addition, a referral centre capable of good quality clinical standards and skin smears, and ability to follow-up patients will be necessary.

The target group as a start will be MB relapse cases as at present it is only secondary drug resistance that the surveillance system will be looking at. Later, as more information on secondary resistance is obtained other risk groups such as defaulters and new cases (for primary resistance) could be considered for inclusion into the surveillance system.

*Definitions and procedures*

Dr. P. Saunderson explained the various categories of relapse, related to the time elapsing since completion of MDT. After discussion the following groups were agreed:

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PB case – relapsing as PB – this group, while common, is not part of the current study

PB case – relapsing as MB at any time:
   New skin lesions (more than 5 lesions in total) and/or a positive BI

MB case – relapsing as MB at any time:
   New skin lesions, and/or an increase in BI of 2 or more units at any single site
   Exclude Type 1 reaction, especially if within 5 years of completion of treatment.

A relapse is defined as the re-occurrence of the disease at any time after the completion of a full course of treatment with WHO recommended MDT. Relapse is diagnosed by the appearance of definite new skin lesions and/or an increase in the bacillary index (BI) of 2 or more units at any single site compared to the BI taken at the same site at a previous examination. Care should be taken to exclude patients suffering from leprosy reactions.

The criteria for inclusion of MB relapse cases in the sentinel surveillance was defined as a person who was initially classified as an MB case and has taken at least 12 monthly doses of MB MDT as recommended by WHO and who is now showing signs and symptoms of relapse and has a BI of $+2$ and above without any evidence of lepra-reaction. A BI of $+2$ was suggested, taking into consideration the present limitations in extracting DNA from tissue specimens with low bacterial load.

Protocol for collection and transporting of samples

The routine procedures practiced in Myanmar in collecting samples and transporting them was presented by Dr. Kyaw Kyaw. At the time of diagnosing a case of relapse at the referral centre a verbal consent was taken from the patient to carry out a slit skin smear as part of the routine management procedure of a case. It was a practice to take slit skin smear samples from four sites where the highest BI was found in the previous examination. There was agreement on the number of sites from which skin smears are to be collected for extracting DNA could be reduced to two sites only. The blade was then placed in the micro centrifuge tube (2 ml O-ring tube) containing 1 ml of 70% ethanol. The tube was then labelled and stored.

The group agreed that the code numbers for samples from the various participating referral centres should start with the country code used in the telephone directory. It was also suggested that the case report forms be typed for easy reading. The time delay in sending specimens to the reference lab is not important technically, but should be minimised for the credibility of the programme.

The specimens are not regarded as infectious and as a result shipment of samples can be done using international courier services. National programmes will be using whatever available courier service that exists in the country for shipment of samples to the reference laboratories.

Laboratory procedures (report of a group discussion)

The following are the main outcome of the group discussion:

- The experts agreed that the number of sites from which skin smears are to be collected for extracting DNA could be reduced to two sites only.
- It was agreed that the stainless steel blade used in taking the skin smear should not be left in the ethanol tube. This is to prevent the blade from getting rusty especially when samples
are stored for long periods as it could interfere with the DNA extraction process later. The skin smear scrapings attached to the blade is to be washed off using the 70% ethanol solution contained in the tube.

- The group also agreed to all use the same PCR procedure and the PCR primers which were designed by Dr. Matsuoka. It was suggested that three genes will be studied, for dapsone, rifampicin and quinolone resistance.

- Experts representing various reference laboratories agreed to provide DNA sequencing results in two separate reports. One report will be provided by the laboratories to the referral centre where the relapse patient is located and where patient’s basic results of the test will be provided. The report will state giving the basic results as:
  
  (a) mutations for resistance to either rifampicin, dapsone or ofloxacin present,
  
  (b) no mutations for drug resistance present, and
  
  (c) no positive PCR products from sample

- The second report will be a more detailed report which is to be sent to the surveillance network, giving the whole sequence. The aim of this is to build up a database on DNA sequencing which would allow monitoring of new mutations.

- Quality control of the participating reference laboratories will be arranged with known samples provided by Dr. Matsuoka and Dr. Stewart Cole. Checking of samples from field laboratories will be done by Dr. Stewart Cole from Global Health Institute, EPFL Department of Immunology, Lausanne, Switzerland.

- Good liaison between sentinel sites and the laboratory is needed. In the case of DNA negative samples, re-sending of specimens may be needed. Discussion about sending additional specimens should be agreed upon on a case by case basis.

Management of relapses with rifampicin resistance (report of a group discussion)

Dr. P. Saunderson introduced the topic on management of relapses. Treatment of relapse cases without proven rifampicin resistance should be treated with standard MDT, whether or not dapsone resistance is present. It was agreed to standardise treatment for cases diagnosed as rifampicin resistant according to the Seventh WHO Expert Committee Report recommendations, using clofazimine, ofloxacin and minocycline. However, minocycline is contraindicated in children less than 12 years, while ofloxacin is also contraindicated in children and adolescents; both are contraindicated in pregnant women. Though the occurrence of drug-resistant leprosy is regarded as a rare event in children under the age of 10 years, as newer drugs for second-line treatment becomes available, appropriate and safe treatment regimens for children and pregnant women must be developed as a priority research area.

Relapse cases provided with second line treatment for drug resistance are to be closely supervised and monitored. The exact mechanism is to be decided by the clinician in charge of treating the case at the referral centre. Frequent contact with the clinician in charge will play a crucial part in ensuring regularity of treatment. However, it was noted that close supervision will depend very much on the local situation including the patient’s ability to travel regularly to the referral centre. If needed, the possible use of initial hospitalisation and/or home visits by the health care workers are to be made available at the referral centre. Referral centres are to be provided with funds to cover patient costs, drugs for second line treatment and supervision purposes.
Dr. Myo Thet Htoon introduced the draft Case Report Form. This form does not include the patient’s name for reasons of privacy. Various details were discussed to simplify the forms further. Based on the recommendations from experts regarding obtaining sufficient samples for DNA sequencing from each relapse case it was agreed that skin smear samples are to be collected from only two sites with the highest possibility of providing high BI. It was agreed that the forms should be tried out and reviewed at the next meeting in light of experience gained in the field.

Sentinel sites currently participating in the global drug resistance surveillance

As the surveillance expands new sentinel sites as well as new reference laboratories are to be added to ensure geographic representation and to increase sample size. The participating reference laboratories agree in principle to test samples from any new sentinel site free
of charge. To ensure quality of the test results it was recommended that in large countries several centres of excellence that have experience in carrying out DNA sequencing studies be selected (Table 1).

CONCLUSIONS AND RECOMMENDATIONS

The following conclusions and recommendations were agreed upon.

1. The revised guideline for Surveillance of Drug Resistance in Leprosy was approved for implementation. Based on further advances in research, revisions to the guidelines are to be made in consultation with all partners and experts.

2. The workshop recommended that the programme of surveillance be started as soon as the agreements from the respective national authorities are obtained. Global Leprosy Programme will collect case reports forms from the participating sentinel sites on a 6-monthly basis. The first round of data collection will be in April 2009 followed by another round in September 2009 issue of the WHO’s Weekly Epidemiological Record. Initial results will be collated and published in the last quarter of 2009 and the findings discussed at the next meeting.

3. Additional sentinel sites are to be included into the surveillance system in order to achieve larger sample sizes and better geographic representations. Similarly, additional reference laboratories are also to be included to improve coverage of the surveillance system.

4. The workshop recommended that a meeting be held annually to review progress and to update recent advances in DNA sequencing technology among all the partners. It was agreed that the dates for the next meeting is to be tentatively set for 26th–27th October, 2009. Venue to be decided later by the Global Leprosy Programme.

Annex 1

Tentative Agenda

<table>
<thead>
<tr>
<th>Monday, 20th October 2008</th>
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<tbody>
<tr>
<td>09:00–09:30 hours</td>
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<tr>
<td>– Welcome by Chairperson</td>
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<td>– Opening address by the Vice Minister of Health, Viet Nam (Dr Nguyen Thi Xuyen)</td>
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<td>– Message from WR Viet Nam (Dr Jean-Marc Olive)</td>
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<td>– Selection of co-chairperson and rapporteur (Dr Myo Thet Htoon)</td>
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<tr>
<td>– Introduction of participants by (Dr Myo Thet Htoon)</td>
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<td>09:30–10:00 hours</td>
<td>Coffee/Tea Break</td>
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<tr>
<td>10:00–10:30 hours</td>
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<tr>
<td>Why we need to monitor drug resistance? (Prof. W.C.S. Smith)</td>
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<tr>
<td>– Discussion</td>
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<tr>
<td>10:30–11:00 hours</td>
<td>Global leprosy situation (Dr Myo Thet Htoon)</td>
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<tr>
<td>– Discussion</td>
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<tr>
<td>11:00–11:30 hours</td>
<td>Summary of recent reports on drug resistance (Dr Matsuoka)</td>
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<tr>
<td>– Discussion</td>
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<tr>
<td>11:30–12:00 hours</td>
<td>Recent advances in DNA sequencing for the detection of drug resistance (Prof. Emmanuelle Cambau)</td>
</tr>
<tr>
<td>– Discussion</td>
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</table>
12:00–12:30 hours  
**Country presentations: Current practices for diagnosis, referral and management of relapses** (20 minutes per country)  
– Viet Nam (Dr Tran Hau Khang)  
– Brazil (Dr Samira Buhrer)  
– Ethiopia (Dr Elizabeth Dizaneh Kassa)  
– India (Dr P.L. Joshi/Dr Rajan Babu and Dr Mannam Ebenezer)  
– Myanmar (Dr Kyaw Kyaw)  

12:30–14:00 hours  
**Lunch break**

14:00–16:00 hours  
Current practices for diagnosis, referral and management of relapses (continued)

16:00–16:30 hours  
**Coffee/Tea break**

16:30–17:30 hours  
Current practices for diagnosis, referral and management of relapses (continued)

**Tuesday, 21st October 2008**

Laboratory aspects: current practices in receiving, processing, testing and reporting on specimen received from the field

09:00–09:30 hours  
Ethiopia: (Dr Beyene)

09:30–10:00 hours  
India: (Dr Rupendra Jadhav)

10:00–10:30 hours  
**Coffee/Tea Break**

10:30–11:00 hours  
Japan: (Dr Masanori Matsuoka)

11:00–12:30 hours  
Brazil: (Dr Philip Suffys)

12:00–14:00 hours  
**Lunch break**

14:00–14:30 hours  
**Discussion on Guidelines**  
Introduction to the objectives and broad outline for Sentinel Surveillance for Rifampicin and Dapsone resistance (Prof. W.C.S. Smith)  
– Discussion

14:30–15:30 hours  
Definition and procedures (Dr P. Saunderson)  
– Discussion

15:30–16:00 hours  
Protocol for collection and transporting of samples (Dr Kyaw Kyaw)  
– Discussion

16:00–16:30 hours  
**Coffee/Tea break**

16:30–17:30 hours  
**Group discussions**  
Group 1. Programme aspects  
Management of relapses with rifampicin and/or dapsone resistance (Dr P. Saunderson)  
Case reporting forms and procedures for data collection, collation, analysis and reporting of results periodically (Dr Myo Thet Htoo)  
– Discussion

Group 2. Laboratory aspects  
DNA sequencing protocol (Dr Masanori Matsuoka and Prof. Emmanuelle Cambau)

**Wednesday, 22nd October 2008**

Discussion on Guidelines (continued)

09:00–09:30 hours  
Report of the group work  
– Discussion

09:30–10:00 hours  
General discussion on future expansion of the sentinel surveillance to other sites

10:00–10:30 hours  
**Coffee/Tea Break**

10:30–11:30 hours  
Drafting of conclusion and recommendations

11:30–12:30 hours  
Finalizing conclusions and recommendations

12:30–14:00 hours  
**Lunch**

14:00–14:45 hours  
Closing of the Workshop
Annex 2

List of Participants

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