Drug resistance surveillance in leprosy

Report of the WHO-ILEP joint meeting
4–6 February 2014
Cebu City, Philippines

World Health Organization
Regional Office for South-East Asia

SEA-GLP-2014.1
Distribution: General

Drug resistance surveillance in leprosy

Report of the WHO-ILEP joint meeting
4–6 February 2014
Cebu City, Philippines

World Health Organization
Regional Office for South-East Asia

Printed in India

Contents

Page

Annexes

Acronyms

AFB	
BI	bacterial index
DDS	diamino-diphenyl sulfone
DNA	deoxyribonucleic acid
DRDR	drug-resistant determining regions
DRS	drug-resistance surveillance
DST	drug susceptibility test
GLP	Global Leprosy Programme
HRM	high resolution melt
IEC	information, education and communication
ILEP	International Federation of Anti-Leprosy Associations
MB	multi-bacillary
MDT	multidrug therapy
MFP	mouse footpad
MoH	Ministry of Health
NCDR	new case detection rate
NLEP	National Leprosy Elimination Programme Headquarters
PCR	polymerase chain reaction
QC	quality control
SINAN	National Information System for Notifiable Diseases
SNP	single nucleotide polymorphisms
SSS	slit skin smear
SVDH	skin and venereal diseases hospital
TAG	WHO Technical Advisory Group on Leprosy Control
TLM	The Leprosy Mission
TNF	The Nippon Foundation
WGS	whole genome sequencing

1. Background

Rifampicin is a strong constituent antibiotic used in multidrug therapy (MDT) besides dapsone and clofazimine. Even 30 years after it was introduced, MDT remains the only WHO recommended regimen for treating leprosy. Emergence of drug resistance to one drug or all the constituents of MDT is reported sporadically in different parts of the world. Surveillance of drug resistance is, therefore, necessary to sustain the gains achieved in leprosy control.

In 2009, WHO published 'Guidelines for Global Surveillance of Drug Resistance in Leprosy'. Currently drug resistance surveillance (DRS) is carried out by screening all multi-bacillary (MB) patients who have relapsed after completing the prescribed WHO MB MDT, on a sentinel-centre basis. The system was developed to detect secondary rifampicin resistance. Drug resistance to dapsone and ofloxacin is also tested simultaneously. Centres having appropriate wherewithal in clinical and laboratory services were identified as sentinel centres. Currently, 17 countries are collecting patient samples for testing drug resistance: Benin, Burkina Faso, Brazil, Colombia, China, India, Indonesia, Madagascar, Mali, Mozambique, Myanmar, Nepal, Niger, Pakistan, Philippines, Viet Nam and Yemen. There are 10 reference laboratories which facilitate processing of these samples for 'leprosy drug resistance DNA mutation' detection. These laboratories are located in Brazil, France, India, Japan, Nepal, Republic of Korea, Switzerland and USA.

The WHO-ILEP joint meeting on drug resistance surveillance was organized during 4-6 February 2014 in Cebu, Philippines to review the progress on drug resistance surveillance in leprosy carried out through the network of sentinel centres. Currently rifampicin resistance does not seem to be a serious problem among relapse cases. Longitudinal observation however, should be continued, alongside primary and other secondary leprosy case surveillance. The situation in leprosy control is not the same as in TB, and vigilance needs to be continued to prevent the occurrence and spread of drug resistance and thus maintain the effectiveness of MDT.

2. Objectives

The objectives of the meeting were as follows:

- to review the drug resistance surveillance data, trends in relapses reported by national programmes and other relevant issues in specimen collection, analysis and reporting;
- to discuss possibilities of integrating sentinel surveillance for drug resistance in leprosy through focal laboratories with existing national drug resistance surveillance systems in high burden countries like Brazil, India and Indonesia; and

- to discuss and define standardizing procedures for inclusion, assuring quality of testing drug resistance and certification of more sentinel centres in drug resistance surveillance.

Expected outcomes

- drug surveillance data from participating sentinel networks presented and analysed;
- trends in relapse cases in participating countries reviewed;
- standard procedures for inclusion, quality assurance and certification developed encouraging wider participation; and
- further collaboration with different surveillance networks and participating laboratories strengthened.

3. Opening session

Dr Marivic Balagon, Executive Director, Leonard Wood Memorial Centre for TB and Leprosy Research, Cebu, Philippines, welcomed the participants. Dr. Ermesto ES Villa Lon III, Leprosy Programme Manager, Philippines emphasized the importance of DRS in leprosy. He reiterated the commitment of the national programme to monitor and prevent drug resistance in leprosy in the country.

Dr Paul Saunderson, Medical Director, American Leprosy Missions, welcomed the participants on behalf of the International Federation of Anti-Leprosy Associations (ILEP) and referred to the following key objectives of DRS through a network of sentinel centres:

- to maintain the integrity of MDT.
- to play an advisory role for both WHO and ILEP on the chemotherapy of leprosy.
- to consider and advise on alternative drug regimens for leprosy
- to encourage collaboration between clinicians, national programme and laboratories.
- to develop a database of leprosy strain types.

Several ILEP members including Association Francaise Raoul Follereau, France, American Leprosy Missions, British Empire Leprosy Relief Association, UK, Damien Foundation, Belgium and Germany Leprosy and TB Relief Association had contributed in strengthening DRS and on their behalf, Dr Saunderson wished all participants successful deliberations.

Dr. Herman Joseph S. Kawuma, Chairman, WHO Technical Advisory Group on Leprosy Control (TAG); Medical Adviser, GLRA, mentioned that though MDT is effective,

there is a need to monitor its effectiveness and prepare for alternative regimens for use if required. TAG is tasked to give advice to WHO, based on available evidence on drug resistance in leprosy and need for new drug regimens.

Dr Sumana Barua, Team Leader, WHO Global Leprosy Programme, said that the meeting would help protect the achievements gained so far since MDT was introduced over three decades ago and facilitate the development of future leprosy treatment strategies.

Dr Roch Christian Johnson, Medecin, Foundation Raoul Follereau, welcomed the participants to the DRS meeting.

4. Setting the stage

Updates on global leprosy situation, implementation of the Enhanced Global Strategy 2011–2015 and sentinel surveillance on drug resistance in leprosy. Dr Sumana Barua,Team Leader, WHO Global Leprosy Programme.

By the end of 2012, the bulk of registered cases of leprosy by WHO Region was concentrated in South-East Asia (125 167; 66%) followed by the Americas (33 926; 18%), Africa (17 540; 9%), Western Pacific (7424; 4%) and the Eastern Mediterranean (4960; 3%). New leprosy case detection followed a similar pattern with 166445 (71%) in South-East Asia; 36 178 (16%) in the Americas; 20 599 (9%) in Africa; 5393 (2%) in the Western Pacific; and 4235 (2%) in the Eastern Mediterranean. New cases are primarily concentrated within 18 endemic countries, with three nations (Brazil, 14%; India, 58%; Indonesia, 8%) reporting 80% of new cases as compared to the rest of the world (*Weekly Epidem Record*, 30 August 2013).

Implementation of the Enhanced Global Leprosy Strategy (2011–2015) in all WHO regions has continued to make significant progress. MDT donated by Novartis is supplied to all endemic countries free of cost. There has been improved programme coverage, strengthening of participation of persons affected by leprosy, and partnerships with national and international organizations resulting in elimination of leprosy as a public health problem in most countries. Initiatives have been encouraged to reduce stigma and discrimination against leprosy-affected persons. The global sentinel surveillance of drug resistance in leprosy (DRS) is operating in selected countries in all WHO regions with regional capacity building workshops.

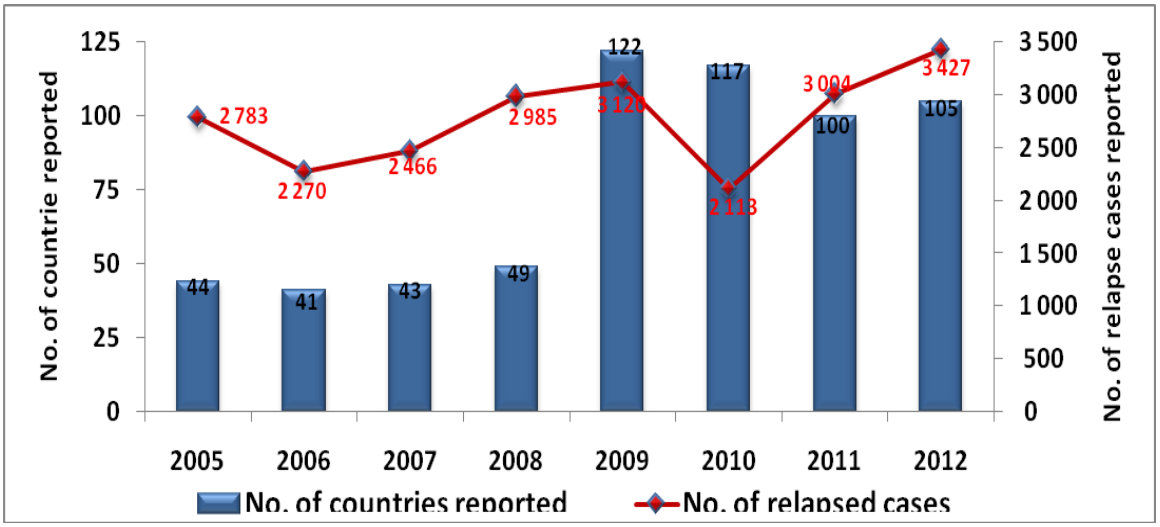
Despite these accomplishments, several challenges as well as priorities for research remain. For instance, improvement is needed in the areas of accessibility to diagnosis and treatment services along with effective referral services with supportive supervision and monitoring at sub-national levels. Effective information, education and communication (IEC) to increase community awareness is needed, as is capacity-building to maintain and yield more effective and functional partnerships at global, regional, national and sub-national levels. Strengthening is needed in the areas of surveillance of drug resistance and community-based initiatives. The International Leprosy Summit was held in Bangkok in

2013, where ministers of health from leprosy-endemic countries at the request of WHO and The Nippon Foundation and other partners discussed reasons for stagnation noticed in leprosy control and reaffirmed political commitment. This resulted in the Bangkok Declaration which noted current challenges and a renewed commitment to further reduce the global burden of leprosy.

The most important aspect of recent trends is that the decline in new case detection which was apparent after 2000, has now ceased and the number of new cases has remained fairly constant since about 2007.

Since the programmes inception in 2008, six annual meetings have been convened. Countries reporting relapse cases have more than doubled since 2008 from 49 to 122, contributing to a pattern of increased relapse reports over the last seven years. In 2012, Brazil reported 1709 (50%) and India 697 (20%) of the 3427 globally-reported relapse cases (see Figure 1 below). The DRS programme is now encouraging surveillance measures to include sampling other secondary as well as primary cases for leprosy drug resistance detection (dapsone, rifampicin, ofloxacin). Better clinical profiles of relapse cases could also provide insight into risk factors.

Figure 1: ***Relapses reported to WHO and number of countries reporting, by year.***



All the reports provide motivational evidence for further drug development. There are few drugs as robust as rifampicin against leprosy. There is a need for suitable patient and trial sites; and the programme would like to encourage a chemotherapy sub-group to improve the management of drug-resistant cases. There is also the clinical question regarding reconciling possible molecular false negatives. At present, resource mobilization for the programme remains a considerable issue, although the programme envisions expansion and inclusion of more endemic countries. No funding is being provided to reporting countries or reference labs to collect and analyse relapse cases according to protocol, which limits the extent of patient reporting that can occur from already funding-strapped NGOs, developing country governments and laboratories dependent on external funding.

Cosidering the limited sampling at present, rifampicin resistance does not seem to be a serious problem among relapse cases. However, longitudinal observation should be continued, alongside primary and other secondary leprosy case surveillance. The situation with referred leprosy control is not the same as in TB, and vigilance needs to be maintained to prevent the occurrence and spread of drug resistance and thus maintain the effectiveness of MDT, as part of the current leprosy control strategy.

5. Country presentations

5.1 Drug resistance surveillance in Benin, Guinea, Mali and Niger

Dr Roch Christian Johnson, Medical Officer, Raoul Follereau Foundation, presented the data from Benin, Guinea, Maliand and Niger. Initially, Benin and Mali were included in this surveillance study. In 2013, Guinea and Niger were also included. From all centres samples were collected and sent to the collaborating reference laboratories in Lausanne and Paris. New case-detection rates per 100 000 from the four countries are: 2.7 for Benin, 3.7 for Guinea, 1.6 for Mali and 2.0 for Niger. Treatment completion rate is >90% in all four countries.

A total of 91 samples (10 from suspected relapse cases and 81 from new cases) were analysed from these countries. Most samples (81 out of 91) were from Benin and Mali. One patient from Mali showed *rpoB* sequences of *M. tuberculosis*, indicating a likely co-infection. They observed the existence of primary dapsone resistance in Benin and Mali. None of the samples showed resistance to rifampicin, but one sample showed a mutation for ofloxacin resistance. Second-line drugs are available at country level for drug-resistant cases.

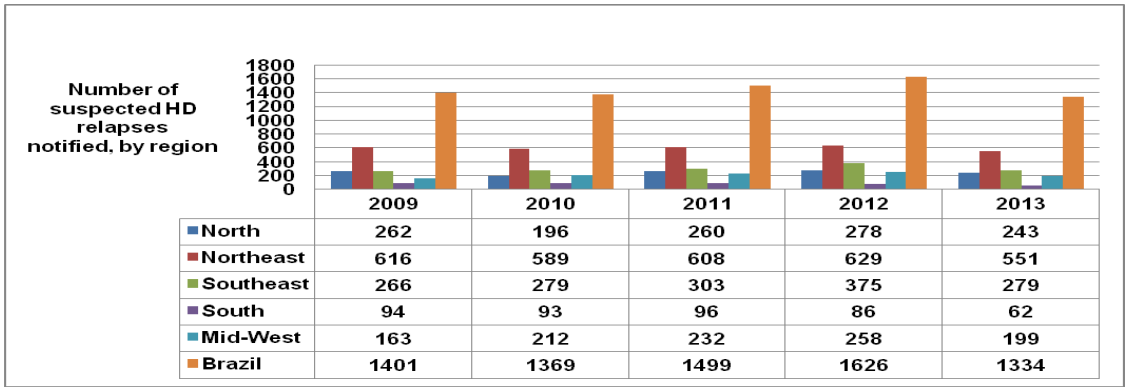
Challenges include the need to increase the number of patients tested, and to include Ivory Coast, as it has >1000 new cases per year.

5.2 Brazil

Dr Rosa Castalia Franca Ribeiro Soares, Coordinator, Leprosy and Diseases under Elimination; Ministry of Health, Brazil said that in 2001, Brazil reported a prevalence of 3.99 per 10 000 population; which by 2012, had decreased to 1.51 with maintenance in spatial patterns of reporting across five higher prevalence states. Between 2003 and 2012 in Brazil, there has been a cumulative reduction in reported new leprosy cases by 41.5%. In 2012, there were 33 303 new cases of leprosy reported of which 6.7% were children (1.717 and 0.48 prevalence, respectively). Brazil recognizes the need to find ways to stop the transmission of leprosy.

In 2013, 1334 suspected relapse cases were reported, roughly maintaining the 2009–2012 trend ranging from 1401–1626 relapses annually (see Figure 2).

Figure 2: ***Number of suspected HD relapses notified by Region***



	2009	2010	2011	2012	2013
North	262	196	260	278	243
Northeast	616	589	608	629	551
Southeast	266	279	303	375	279
South	94	93	96	86	62
Mid-West	163	212	232	258	199
Brazil	1401	1369	1499	1626	1334

Of the relapse cases in 2012, 68.7% were checked by slit skin smear (SSS) (40.4% were AFB positive 28.3% were AFB negative); SSS was not performed for 22.1% cases and 9.2% of patients had no SSS information available. After clinical confirmation of relapse, cases were referred for advanced analyses including serology, SSS, skin biopsy and Shepard mouse foot pad (MFP) analyses by one of four local reference centres assigned coverage of different regions: Instituto Lauro de Souza Lima, Bauru, Sao Paulo, Foundation Alfredo da Matta (Manaus, Amazonas), Centro de Referencia Nacionalem Hanseniase/Dermatologia Sanitaria Uberlândia, Minas Gerais, and the Oswaldo Cruz Foundation Institute Rio de Janeiro. SSS sample collections are stored in ethanol until isolation for DNA analyses. Of drug-resistant positive samples, 10% are also forwarded to the Japanese referral laboratory within the DRS quality control programme. All participating centres are Brazilian government facilities, supported by state or national level support activities and using their own resources to process or send patient samples to referral sites.

Dr Patrícia Sammarco Rosa, Instituto Lauro de Souza Lima, Bauru/SP, Sao Paulo said that in 2013, 99 leprosy patients were examined by SSS (73) and/or skin biopsy (57) and processed by MFP and/or direct sequencing at ILSL. Of the SSS samples, no resistance was detected to dapsone, but resistance to rifampicin was found in 12 (16.4%), of which eight were relapse cases and four were new cases, with 34 (46.6%) demonstrating no amplification. Of the 12 rifampicin-resistant cases, none were relapses, eight were secondary and four were primary cases. Of the biopsy samples, 48 were relapse cases with 30 (57%) sensitive, one (2%) dapsone resistant, one (2%) rifampicin-resistant and one (2%) resistant to quinolones. Mutations were detected at rpoB 451 (CAC-TAC; His-Tyr), folP1 55 (CCC-CTC; Pro-Leu) and gyrA 91 (GCA-GTA, Ala-Val), with some individual patients reporting more than one type of resistance. All cases in which resistance was detected were treated with clofazimine, ofloxacin and minocycline. Three new and six other secondary cases were also tested, but all were drug sensitive.

Last year, a small outbreak of 16 cases of drug resistance (including nine relapse cases and seven new cases) was reported in a population that was once a leprosy colony but is now more of a general community. A thorough investigation has been initiated in recent months; however, drug resistance profiles are still in progress.

The Foundation Alfredo de Matta, Manaus Ama Zonas, has analysed 191 cases for drug resistance over the period 2007–2013, of which 89 relapse cases yielded two (2.2%) dapsone-resistant, five (5.6%) rifampicin-resistant, and two (2.2%) quinolone-resistant cases. Two of these relapse cases were multiple resistant. Of 102 new leprosy cases tested, two (1.9%) were rifampicin-resistant. It is not known if this is true primary rifampicin resistance or whether it followed treatment for other infectious diseases, such as TB.

Dr Philip Suffys, Head of the Laboratory of Molecular Biology, Oswaldo Cruz Foundation Institute, Rio de Janeiro said that in 2013, he had studied 27 relapse cases, with no resistance found. Since 2009, 21 relapse cases have shown mutations in gyrA A19V; however, the mutation is thought to be downstream of the resistance region. Rifampicin resistance has been shown in a few cases in rpoB 531 and 526.

Recent TB research indicates that patients can be hetero-resistant or harbour both drug-resistant and sensitive strains of *M. tuberculosis* (Folkvardsen et al, Streicher et al). In such cases, molecular methods such as line probe assays or sequencing do not always detect clinically-relevant resistance, which can be more sensitively detected by conventional drug susceptibility assays (ie, culture). In regard to leprosy, this implies that clinically relevant hetero-resistant cases may not be detectable by current molecular methods alone. The relevance of hetero-resistance may be even more challenging to explore in leprosy as access to MFP culture has become more limited. Digital polymerase chain reaction (PCR) techniques are currently under development for improving detection and quantification of hetero-resistance (Pholwat et al.) which, if similarly applied to leprosy, may result in greater sensitivity for resistance detection.

During the discussion, the importance of differentiating between suspected relapses and true relapses was pointed out; cases should be investigated as thoroughly as possible, although even experienced clinicians cannot always make a definite decision. Clearly, the country-level relapse figures reported to WHO represent a wide range of clinical cases.

In summary, 20 cases of rifampicin resistance were detected in Brazil in 2013. Of these, 14 were relapse cases and six were new cases.

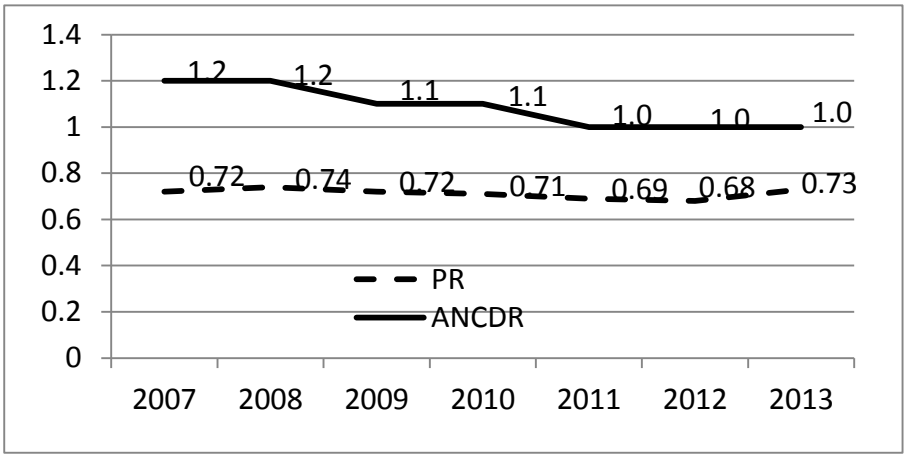
5.3 Colombia

Dr Juan Camilo Beltrán-Alzate of the Instituto Colombiano de Medicina Tropical – Universidad CES Fernando López, Sanatorio Agua de Dios, presented the data on drug resistance surveillance in Colombian leprosy patients, 2012–2013. Leprosy elimination was achieved in Colombia in 1997. New case detection rates have remained relatively stable with 434 new cases reported in 2013 with a prevalence rate of 0.16 per 10 000 population. During 2012–2013, sentinel surveillance through a university laboratory involved working with 10 different local departments to sample 207 patients and 357 household contacts. In 2012, 49 relapse cases were identified; resistance was detected in one MDT defaulter case: rpoB S456L. Challenges to continuing surveillance are financial support as well as contact with a reference laboratory for quality control, training, sequencing, etc.

5.4 India

Dr C M Agrawal, Deputy Director-General (Leprosy), informed about the leprosy situation in India. New case detection and prevalence have reached a plateau, as shown in Figure 3, with around 130 000 new cases detected in each of the last five years.

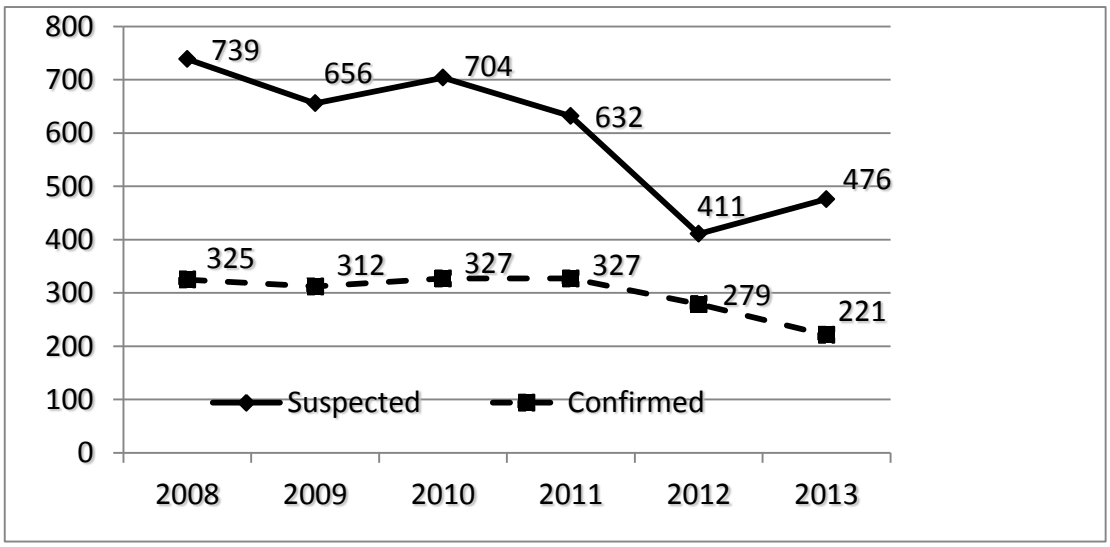
Figure 3: ***Prevalence rate and annual new case detection rate, per 10 000 population, in India***



Source: WHO GLP

The current strategy for leprosy control includes decentralized and integrated services, treatment with MDT and examination of household contacts. Treatment completion was 93.8% in 2012–2013. Over 13 000 reconstructive surgery procedures to correct G2D were performed in the last five years.

Figure 4: ***Relapse cases in India***



Source: WHO GLP

Figure 4 shows the number of relapse cases suspected at the primary level (new skin lesions after over three years of completing MDT) and confirmed at secondary or tertiary level by various methods, such as skin smear, therapeutic trial of steroids and histopathology. The states with the highest numbers of relapse cases were Maharashtra (44 cases) and West Bengal (51 cases). Uttar Pradesh had the highest number of suspected relapses (123), but only 21 were confirmed.

For drug resistance surveillance, 12 states were identified as priorities and links with the three laboratories in the programme are being established: National JALMA Institute, Agra; Blue Peter Research Centre, Hyderabad; and Stanley Brown Laboratory, The Leprosy Mission (TLM) Delhi. Up to now, the laboratories have been working on their own, but it is proposed to link them with the National Leprosy Elimination Programme (NLEP) in a national sentinel surveillance programe, using WHO protocols.

Dr J Subbanna presented data from the Blue Peter Research Centre, Hyderabad, covering relapse cases in Andhra Pradesh and Odisha during one year (2012–2013), as shown in Table 1.

Table 1: *Data on relapse cases*

	Andhra Pradesh	**Odisha**	**Total**
New cases	8295	8226	16521
MB cases	4072	4098	8170
Treatment completion rate	96%	98%	97%
Relapse cases	48	27	75

Source:

In total, 29 relapse cases were investigated for drug resistance, but only nine samples were PCR positive. In these nine samples, no mutations were identified in the *rpoB* and *gyrA* genes; studies of the *folP* gene failed on this occasion. In the past (2010), two samples with *folP* mutations were identified in samples from Odisha.

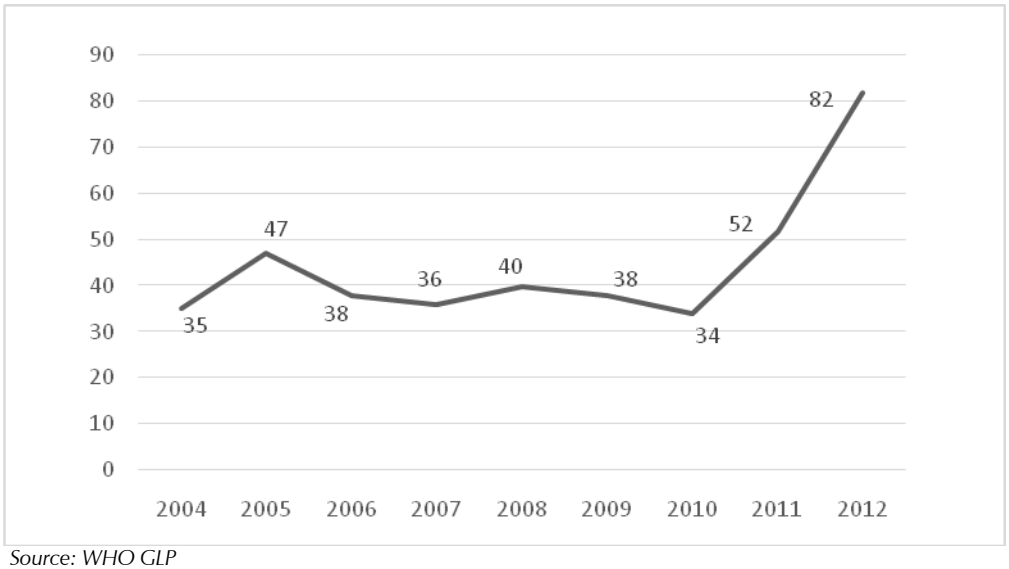
Funding for transport and sequencing (which is outsourced) remains a challenge, and there is a need for further training of field staff.

In discussion, it was pointed out that many samples had a low BI (of the 29 samples tested, only 17 had a BI of two or more and only six had a BI of four or more), so this may account for the low number of samples which gave a positive PCR result.

Dr Mallika Lavania, Research Scientist, presented data from the Stanley Brown Laboratory, Delhi. They received samples from five TLM-India hospitals around the country. Over 5000 new cases are registered annually in the TLM hospitals, with a gradually increasing percentage of MB cases, currently at 78%. Around 25% of all new cases have a positive skin smear, and half of these have a BI of four or more.

Reported relapse cases have increased this year, as shown in Figure 5. Samples from 53 relapse cases were sent for analysis at SBL, although 12 samples had a BI of less than 2+. Out of 44 samples tested for dapsone resistance (*folP*gene), one mutation was found; of 43 samples tested for rifampicin resistance (*rpoB* gene), seven with mutations were found; of 46 samples tested for ofloxacin resistance (*gyrA* gene), two with mutations were identified. While ofloxacin resistance is less than last year (five cases found), this is the first time any rifampicin resistance has been found at SBL. Twenty six additional cases of relapse were identified in a community in West Bengal, near Purulia hospital, and three of these showed rifampicin resistance, making in total of 10 cases this year.

Figure 5: ***Relapse cases identified at TLM hospitals in India.***



Source: WHO GLP

The challenge within TLM is to motivate staff at all 19 centres to identify relapse cases and send samples for analysis.

Mr Sundeep Chaitanya, Research Officer presented an analysis of a possible new mutation in the *rpoB* gene, carried out at SBL. They identified a mutation not previously described (Gln 442 His) in patients 4, 8 and 9, as indicated in Table 2 below. High resolution melting (HRM) curves suggested that this mutation behaved like a resistant mutant.

They built computer models of the wild-type RNA polymerase B protein and the protein with this new mutation, showing that the new mutation did appear to alter the rifampicin-binding region of the gene, making it more difficult for rifampicin to bind to the molecule.

In discussion, it was agreed that new mutations need to be assessed to show whether they confer resistance or not, as there are many examples of mutations that are not associated with resistance. The easiest way to test such mutations is to introduce the altered gene into *M Smegmatis*, which can then be subjected to simple drug-susceptibility

tests in the laboratory Dr. Williams said that it is very important to preserve these new strains of *M leprae* so that they can be studied further.

Table 2: *Mutations in the rpoBgene in 10 relapse cases studied at SBL*

Case No.	Location	Timing of Relapse (years)	Age/Sex	BI		Mutation
				Previous	Current	
1	Purulia	15	68/M	NA	4+	**Gly 495 Ser**
2	Purulia	10	42/M	5+	4+	**Thr433Ile; Asp441Tyr**
3	Purulia	5	51/M	4+	4.66+	**Glu471Lys; Val475Met**
4	Shahdara	NA	20/M	3+	3+	**Gln 442 His**
5	Shahdara	20	37/M	NA	4.33+	**Gly 462 Del**
6	Shahdara	5	30/F	NA	4+	**Thr433Ile; Asp441Tyr**
7	Shahdara	5	46/M	1.66+	2.66+	**Pro 460 Del**
8	Kolkata	6	35/M	NA	5+	**Gln 442 His**
9	Kolkata	5	40/M	NA	3+	**Gln438Val; Gln 442 His**
10	Kothara	6	40/M	NA	4+	**Phe439Leu; Gly462 Del**

Source:

In conclusion, it is noteworthy that the NLEP is fully supportive of the programme of surveillance for drug resistance in leprosy and that the process for testing *M leprae* strains for resistance is well established. For the first time, a significant focus of rifampicin-resistant cases has been identified this year in West Bengal, and it is important that the situation is closely monitored. Ideally, any multibacillary cases in the area near this focus should be studied, to examine the extent of drug resistance.

5.5 Indonesia

Dr Christina Widaningrum, Head, Subdirectorate of Leprosy, Ministry of Health presented data for Indonesia, with 17 000–20 000 new cases per year over the last five years, giving a NCDR of 7.76 per 10^5 and a PR of 0.91 per 10^4, in 2012. Relapse cases have increased from 142 in 2010 to 192 in 2012. High burden areas include Java, Sulawesi, Maluku and Papua; the highest reported new case detection rate (NCDR) is in West Papua (72 per 10^5).

Suspected relapse cases are referred to sub-sentinel centres for confirmation and 10% of confirmed relapse cases are sent to the Tropical Disease Centres, Surabaya, for DNA sequencing.

In 2012, 40 samples were examined: for the *folP* gene, six samples could not be read, 32 had no mutation and two showed DDS resistance; for the *rpoB* gene, 14 samples could not be read, 25 had no mutation and one showed rifampicin resistance.

In 2013, 200 samples were examined; 4 of 153 readable samples showed a *folP* mutation; 1 of 148 readable samples showed an *rpoB* mutation; and 0 of 158 readable samples showed a *gyrA* mutation.

In future, it is planned that drug resistance surveillance will be part of the 2015-2019 strategy and second-line drugs will be made available for cases with proven drug resistance.

It is noteworthy that Indonesia is implementing chemoprophylaxis with single-dose rifampicin in household contacts in a remote endemic district.

Type	Number of biopsies	Results
New cases	53	20 cases : still in process 33 cases : 23 sensitive 8 negative (PCR) **1 rifampicin resistant** **1 dapsone resistant**
Relapse	06	4 sensitive to all three drugs 1 : negative (PCR) 1 : in process

Source: WHO GLP

5.6 Madagascar

Dr Andriamira Randrianantoandro, Chief, Leprosy Region presented data from Madagascar, with a population of 21 million. New case detection is more than 1500 cases per year (7 per 10^5), with a G2D rate of 19%. Treatment completion was reported at 93%.

Relapse cases reported were eight in 2010, 26 in 2011, 17 in 2012 and 14 in 2013. It was only in 2013, however, that such cases were investigated and tested for drug resistance in the Lausanne Laboratory. Six health centres have been designated as sentinel sites and they were able to take biopsies from six relapse cases and 53 new cases.

Results are shown in Table 1. There is a need to improve the investigation of relapse cases. Challenges include the difficulty of travel within the country.

5.7 Nepal

Dr Chudamani Bhandari, Director, Leprosy Control Division, Department of Health Services, Ministry of Health and Population, Kathmandu, Nepal described the demographic situation of Nepal. The total population (census-2011): 26 494 504, urban

population: 4 523 820 (17.1%), population growth rate (census-2011): 1.4%, fertility rate (NDHS-2011): 2.6%, literacy rate (census-2011): 65.9% life expectancy: 62.2 years.

Leprosy elimination was declared on 19 January 2010. The current situation is: total new cases – 3253, of which >86% is contributed by the Terai districts (14/75 districts with PR >1). More than 90 % districts reported leprosy cases. A total of 172 000 cases (cumulative) have been treated by MDT. Stigma, chronic ulcers and disability due to leprosy affect more than 30 000 individuals. Eighteen relapse cases were reported in 2013.

The major achievements last year included sustained elimination at the national level, a prevalence rate reduced from 0.85 to 0.82/10 000 as compared to last year, and a new case-detection rate reduced from 1.22 to 1.19/10 000 population, compared to last year. The G2D 2 rate increased to 3.16% from 2.89% in 2012. The cure rate was over 95 % for PB cases, and over 90 % for MB cases.

Dr Deanna Hagge, Head of Laboratory, Anandaban Hospital, Nepal presented the data on drug resistance surveillance including a brief historical overview. Anandaban Hospital was established in 1957 and leprosy surveys started in 1960. In 1966, a small project with dapsone monotherapy was started. Anandaban began MFP studies for dapsone resistance in 1980 and both primary and secondary dapsone resistance were detected. MDT was introduced in 1982 in Nepal and coverage reached 100% in 1996. Elimination was declared in 2010 and Anandaban joined the WHO Global Surveillance of Leprosy Drug Resistance.

There are three leprosy referral hospitals in the country; Anandaban (near Kathmandu), Green Pastures (Pokhara), and Lalgadh (southern plains or Terai). In 2013, the Nepal Leprosy Control Division notified all leprosy care providers that suspect relapse cases were to be referred to Anandaban for confirmation.

A total of 37 relapse cases were reported. Among these, 28 had received MB-MDT, eight had received PB-MDT and one had received dapsone monotherapy. SSS/biopsies were collected from the leprosy patients having BI 1+/>2+. Biopsies were sent to Karigiri for histopathology and inoculated into mice for MFP studies. After extracting DNA from biopsies/SSS, PCR was done for *rpoB,folP* and *gyrA*, and PCR amplified products were sent to another laboratory for DNA sequencing. While 88% of prior MB cases were histologically BL/LL on relapse and 72% of prior PB cases were also histologically BL/LL relapses. A total of 16% of all cases had reaction at relapse. Age at relapse was not significantly different in MB and PB relapses. Many PB relapses were likely cases of misdiagnosis/under-treatment. The time period from RFT to relapse was not significantly different in PB/MB relapses. In 2012–2013 they reported one sample having resistance to rifampicin at codon position 419 (Thr to Ile). Along with this, they have reported 66 secondary relapse cases since 2000. Among these 66, 13 (12.5%) were reported at relapse with reaction 7 T1R, 6 ENL, MFP viable and/or histologically active.

In 2013, 71% of the patients relapsed ≥ 10 years RFT (1-33yrs) and 97% were histologically active. The time to relapse was significantly shorter for 12 doses MB MDT

compared DDS Mono therapy ($p<0.01$) but relapse time did not significantly differ from other treatments; average time to relapse was 13 years. In 2012–2013 no drug resistance mutations were detected at recognized sites.

The support was provided by the Nepal Leprosy Control Division, non-govermental leprosy control partners, Colorado State University (USA), and Stanley Browne Laboratories, Delhi for sequencing facility.

5.8 Philippines: Drug resistance in leprosy, (Leonard Wood Memorial Center) Phillipines

The Leonard Wood Memorial Centre serves as a DRS sentinel site in the Philippines. However, referral of cases and transport of samples from satellite centres to the sentinel site is very restricted. Establishment of additional sentinel sites has been suggested. A total of 19 leprosy cases were monitored in 2012. None of the samples were found to be resistant to rifampicin and ofloxacin. However, dapsone resistance was noted in one untreated case. A total of 21 cases were enrolled in 2013. Molecular drug resistance findings are pending. Based on the drug resistance findings, The Leonard Wood Memorial Centre relapses were not due to drug resistance but were either due to reinfection or persister organisms. Though not statistically significant, findings of LWM suggest the long-term efficacy of the WHO-MDT. Dapsone resistance was detected in one untreated case. There is no cause for concern because of the bactericidal efficacy of the other two MDT component drugs namely, rifampicin and clofazimine.

5.9 People's Republic of China

Dr ShenJianping, Deputy Director, National Centre for Leprosy Control said that MDT was introduced in 1982 with total coverage achieved since 1986. Currently, there is a reported 98% MDT completion rate. Most new cases in 2012 were SSS positive (65.6%) and received WHO recommended MB MDT (82.2%). Distinctive in comparison to many other national programmes, Chinese patients are only released as cured from the active case register after they have completed MDT, disappeared after active skin lesions including reactions and neuritis; and after negative SSS results are achieved during annual follow-ups. In practice, PB patients usually experience three years of follow-up on the active case register and MB patients, 5–6 years; after which patients are transferred to an inactive patient register and seen every 2–3 years by county-level health workers. Relapse is reported by county-level health or paramedical workers. Also, relapses may be found during active case-finding activities (surveys, household contact exams, etc). Relapse confirmation criteria include the reappearance of new symptoms and signs of disease after MDT completion with any one of the following: positive SSS after recorded negative SSS follow-up, an increase of at least two bacterial index (BI) points at any previous SSS site, or skin biopsy histological change and significant solid AFB.

Thirty seven suspected relapse cases were reported in 2012, of which 27 were confirmed. Most (20) were originally SSS positive, all became SSS negative during follow up and then all became SSS or biopsy positive for *M. leprae* upon relapse, at a mean of

15 years after RFT (range 1-28 years). Fifteen of the 27 cases developed new disability (55.6%). Relapse occurred across the Ridley-Jopling spectrum: TT (5), BT (2), BB (3), BL (10), LL (7). Male: female ratio was 20:7 with the average age of relapse at 51 years. Thirteen of the 27 confirmed relapses came were from one province, Yunnan.

Professor Wang Hongsheng, Associate Professor, National Centre for Leprosy Control, China said that in 2013, 925 new cases and 65 relapse cases of leprosy were reported in China, of which roughly half were MDT relapse. Sentinel surveillance for leprosy drug resistance covers 15 key provinces in southern China, and samples are processed according to WHO programme protocols. In 2013 seventeen relapse cases and nine patients with persistent high BI were analysed. Dapsone resistance was detected in one relapse case: *folP* 55 (CCC-CTC; Pro-Leu), but no *rpoB* or *gyrA* mutations. Collection of samples from all relapse cases remains a challenge; however, the national programme has been able to obtain permission to investigate new and relapse cases for the whole of China for the next three years.

5.10 Mozambique, Myanmar and Viet Nam

Dr M Kai, Researcher, National Institute of Infectious Diseases, Tokyo presented on behalf of the above national programmes, results from these countries. There are two sentinel sites in Viet Nam, two in Myanmar and seven in Mozambique; samples are sent for analysis to the Leprosy Research Centres, National Institute of Infectious Diseases, Tokyo, Japan.

In Viet Nam, there was only one relapse case in which no mutation was found. Sixteen new cases were also investigated and one mutation in the *rpoB* gene was found.

In Myanmar, 19 relapse cases were studied. No mutations were found in the *folP*and*gyrA* genes, but two cases showed drug resistance mutations in the *rpoB* gene. Another case had an *rpoB* mutation that is not known to produce drug resistance.

In Mozambique, 11 relapses were investigated, with no mutations in the *folP*and*gyrA* genes, and one mutation in the *rpoB* gene.

5.11 Yemen

Dr Abdul Rahim Al-Samie, Director, National Leprosy Elimination Programme presented information from Yemen, which is considered a low-endemic country. There were 383 new cases in 2013 (MB 56%) and the new case-detection rate is 1.6 per 10^5 population, while the prevalence rate is 0.17 per 10^4. Children comprise 12% of new cases and 7% have G2D at diagnosis. Although the programme is integrated, primary health care is weak and there is a need for strong vertical support from the NLEP, which is supported by WHO, GLRA and YELEP, a local NGO.

Relapses are few, 2–6 cases per year, with two in 2013. The two relapse cases and 3 new cases were investigated for drug resistance through the Lauranne Laboratory. No mutations were found.

It was noted that rifampicin is widely available in the open market in Yemen, so primary resistance may be expected, although it was pointed out that one did not know for sure.

There was a discussion about the time expected between RFT and the occurrence of relapse. Normally, it is expected to be a minimum of 3–5 years, but it is not an absolute rule and therefore, cannot be part of the definition of relapse. It was pointed out that reactions are much more common than relapses and should therefore, be considered the most likely cause of new or active lesions in cured patients. Even in a reinfection, it would take several years for the bacilli to multiply and cause overt disease.

6. Technical sessions

6.1 Drug Resistance in leprosy patients

Dr Diana L Williams, National Hansen's Disease Programme presented a retrospective drug susceptibility testing (DST) study of 61 patients whose specimens were obtained at the NHDP during 2010–2012. The majority of specimens were obtained prior to MDT from patients who had origins in the US or in 10 other countries. Of these, 20% of these patients had PB disease and 80% had MB disease. All specimens were positive for *M. leprae* DNA in *M. leprae*-specific RLEP Real-Time PCR assay. Of these, 48 (~79%) gave sequence results for all three drug resistance determining regions (DRDR) for dapsone, rifampin and ofloxacin. However, only 3% of PB patients gave DST results. Two MB patients had mutations in the folP1 (DRDR) associated with high-level dapsone resistance and one of these patients also had contained a mutation in the rpoB drug resistance determining regions (DRDR), associated with high-level rifampicin resistance. Both pre-treatment and post-treatment biopsies from this patent contained the same DRDR mutations and SNP strain type; strongly indicating that this patient had relapsed with primary multi-drug resistant leprosy. Taken together these results suggest that presently drug-resistant leprosy is not a major problem for leprosy control in the USA.

DST for all three DRDRs were obtained from 48 patients from 2010–2012. Three patients had dapsone-resistant *M. leprae* (6%) and one of these patients also had rifampcin-resistant *M. leprae* (2%). All mutations identified were previously associated with either dapsone or rifampicin resistance. The patient who had both dapsone-resistant and rifampin-resistant (multidrug-resistant) leprosy was a relapse case from Hawaii with origins in American Samoa. Both pre-treatment biopsies and post-treatment biopsies contained the same DRDR mutations and SNP strain type strongly indicating that this patient had primary multi-drug resistant leprosy. This is the first case of primary MDR leprosy in the patient population in the USA.

6.2 Whole Genome Sequencing (WGS): further steps in drug resistance surveillance

Professor Stewart Cole described the applicability of this technique in reference to drug resistance surveillance. Sequencing of *M. leprae* genome was initiated in 1991 and completed in 2001 (Cole et al 2001). The genome sequence was of a strain of *M. leprae*, originally isolated in Tamil Nadu and designated TN. This whole genome sequencing revealed the characteristics of *M. leprae* genomic structures. The *M. leprae* genome is circular and its size is 3.3 Mb compared with 4.4 Mb for *M. tuberculosis*. The *M. leprae* genome possesses 1614 open reading frames and contains 1300 non-coding or pseudogene sequences.

He then described the single nucleotide polymorphisms (SNPs) which are very rare at 1 per 28400 base pairs. On the basis of three SNPs in *Mycobacterium leprae*, there are four major SNP types associated with different geographic regions around the world (Monot et al, 2005). By using comparative genomics, they demonstrated that all cases of leprosy were attributable to a single clone whose dissemination worldwide can be retraced from SNP analysis. Over 20 sub-types can now be identified through SNP analysis. Leprosy seems to have originated in Eastern Africa or the Near East and spread with successive human migrations. Europeans or North Africans introduced leprosy into West Africa and the Americas within the past 500 years (Monot et al, 2009). Leprosy reached America from across the Atlantic Ocean, not via the Bering Straits. By using these SNPs, they observed a strong association between geography and genotype.

With the development of newer technology, the analysis of the whole genome has become cheaper, with results available in less time and through a less cumbersome process. The cost per sample is around US$ 1000 and decreasing. *M. leprae* genome analysis now takes one week rather than one year as previously and requires a much smaller amount of DNA, making it possible on an ordinary biopsy sample.

In collaboration with Dr Matsuoka and Dr Masanori Kai, the whole genome of one MDR strain, Airaku 3 having a folP mutation, was performed (Maeda et al 2001). Airaku 3 has SNP type 1D, and 14 unique SNPs, two of which are in transporter genes ctpC and ctpI. SNP3262657 C>T&SNP953582 C>G give a better typing system: SNP Subtype1D1 was reported from South America and 1D2 from India, Bangladesh, Pakistan and Japan.

Prof Cole also presented his findings about ancient leprosy. Leprosy was endemic in Europe until the Middle Ages and in the twelfth century, there were said to be around 19 000 leprosaria; it is unclear why leprosy disappeared after that period. Using DNA array capture, they obtained genome sequences of *M. leprae* from skeletons of five medieval leprosy cases from the United Kingdom, Sweden, and Denmark. Whole genome sequencing was done from bone and teeth of the skeletons from the above mentioned places. The ancient *M. leprae* sequences were compared with those of 11 modern strains, representing diverse genotypes and geographic origins. The comparisons revealed remarkable genomic conservation during the past 700 years, a European origin

for leprosy in the Americas, and the presence of an *M. leprae* genotype in medieval Europe now commonly associated with the Middle East (Schuenemann et al 2013).

Prof Cole mentioned that the methods which we are using nowadays for drug resistance surveillance are accurate, but laborious. He presented the data on drug resistance in 56 samples from Benin, Guinea, Madagascar, Mali, Niger, Switzerland and Yemen. None of the samples showed mutation in any of the target genes (rpoB, folP and gyrA). In conclusion, he emphasized the application of WGS technique as:

- capture arrays to replace PCR-based molecular drug susceptibility testing;
- providing drug susceptibility and the genome sequence for the same cost and effort; and
- epidemiology at single nucleotide resolution, which could give further insight into transmission.

During discussion Dr Saunderson asked about comparison of VNTR data with WGS data for epidemiological purposes. Prof Cole replied that as VNTRs are highly variable we cannot rely on them for a coherent picture of leprosy epidemiology.

6.3 Review of past quality control studies in the DRS and criteria for inclusion of reference centres.

Dr Masanori Matsuoka, National Institute of Infectious Diseases, Leprosy Research Centre, Tokyo, Japan said that in a quality control assessment in 2010 to evaluate the capability of analysis, samples of bacillary suspension and DNA were sent for analysis to the 11 reference laboratories within the DRS programme. Four *M. leprae* strains were sent from this laboratory to assess yields of template DNA, efficacy of DNA preparation, PCR sensitivity/specificity, and accurate sequencing. Eight of the laboratories responded with results: three laboratories demonstrated a little low PCR sensitivity, three laboratories reported discordant sequence results, but no negative controls produced false positives in any of the laboratories.

Later in 2010, another quality control assessment was performed by distributing template DNA to 17 DRS reference laboratories in nine countries. Thirteen laboratories responded with results, only two of which demonstrated unsatisfactory PCR sensitivity (one of which also reported similarly in the first round of quality control).

In a third round of quality control, *M. leprae* suspensions were provided to evaluate the efficacy of DNA extraction by each laboratory, using their own preferred DNA extraction procedures. Results indicated that template DNA was prepared efficiently at almost all reference centres regardless of the protocol applied at each laboratory.

In this meeting, it was agreed that quality control should be maintained and extended to other laboratories that have joined since 2010. Currently, the DRS programme has sentinel sites for sample collection in 20 countries (Benin, Brazil, Burkina Faso, China, Colombia, Côte d'Ivoire, Ethiopia, Guinea, India, Indonesia, Madagascar,

Mali, Mozambique, Myanmar, Nepal, Niger, Pakistan, Philippines, Viet Nam and Yemen) and reference laboratories in nine countries (Brazil, China, France, India, Indonesia, Japan, Nepal, Switzerland and USA). Qualifications for a reference laboratory should include high yield of template DNA from samples, efficacy of DNA preparation, PCR sensitivity/specificity, and accurate sequencing.

6.4 Management of multi-drug resistant cases of leprosy

Prof Emmanuelle Cambau, University Paris Diderot, Saint Louis-Lariboisière Hospital, National Reference Centre for Mycobaceria and drug resistance, Paris, France stated that Multi-drug resistance in leprosy is currently defined as resistance to both rifampicin and dapsone. Molecular detection of rifampicin resistance correlates with recognized mutations in eight codons within the region determining rifampicin resistance in the subunit B of RNA polymerase encoding gene (*rpoB*): 432 (Gly to Ser), 433 (Thr to Ile), 436 (Leu to Pro), 438 (Gln to Val), 441 (Aps to Asn or Tyr), 451 (His to Asp or Tyr), 456 (Ser to Leu, Met, Phe, or Trp), and 458 (Leu to Val) (Honore 1993, Maeda 1999, Maeda 2001, Cole 2001, Cambau 2002, Zhang 2004, Cambau 2012). Three other RDRR mutations have been reported but remain to be validated as functionally resistant: 411 (Ala to Thr), 505 (Arg to Trp), and 442 (Gln to His) (Colombia 2012; SBL 2013). Dapsone resistance is known to correlate with mutations detectable in 2 codons within the region determining dapsone resistance (RDDR) in the dihydropteroate synthase encoding gene (*folP1*): 53 (Thr to Ile, Arg or Ala) and 55 (Pro to Arg or Leu) (Kai 1999, Gillis 2000, Cambau 2006). Another two mutations have also been reported, but remain to be validated: 88 (Thr to Pro) and 91 (Asp to His) (Nora Cordona, Cotonou 2012).

Within the DRS programme, the French reference laboratory has analysed 170 samples collected from seven sentinel sites since 2009: Benin, Burkina Faso, Ethiopia, Madagascar, Mali, Niger and Pakistan. Three of these countries yielded cases with resistance detectable by mutations: Benin (1 fluoroquinolone resistant, A91V), Mali (3 dapsone resistant, P55L (1) and T53R(2)).

Various controls for these molecular tests in regard to clinical relevance have been discussed. If PCR is negative, alternative checks could be made by BI, RLEP PCR or increasing the analysis concentration of the *M. leprae* DNA. If positive PCR and sequence analyses combine to detect a mutation, a second confirmation analysis might be performed. If the mutation is known to confer resistance, it should be reported in a timely manner to the supervising clinician. If the mutation is not known to confer resistance or is rare, the mutation should be confirmed in another specimen or in another expert lab before reporting to the clinician.

From 2008 to 2010, the DRS programme has increased from three reporting countries to eight, encompassing 1055 reported relapse cases of which 360 (30%) were studied and 60% of those were PCR positive (WHO weekly epidemiological record, 2011). Confirmed resistance was detected for dapsone (29 cases), rifampicin (16 cases) and fluoroquinolones (2 cases). Among those tested, therefore, rates of positive resistance detection were 15% dapsone, 7% rifampicin and 1% fluoroquinolone resistance in

secondary cases. Similar profiles for 2011 and 2012 remain to be compiled. At this meeting, 37 cases of confirmed *rpoB* mutations correlative to resistance were reported across nine countries: Brazil (20), Colombia(1), India (7), Indonesia (2), Madagascar (1), Mozambique(1), Myanmar (1), Nepal (3) and Viet Nam (1).

For cases with detectable rifampicin resistance, WHO recommends that they receive a two year regimen of six months of three daily drugs including 50mg clofazimine, 400mg ofloxacin and 100mg minocycline or 500mg clarithromycin followed by 18 months of two daily drugs including 50 mg of clofazimine with 400mg ofloxacin or 100mg minocycline. Susceptibility to second line drugs ofloxacin, minocycline and clarithromycin should also be considered. There are two codons within the region determining fluoroquinolone resistance (RDFR) in the A subunit of DNA gyrase encoding gene (*gyrA*): 89 (Gly to Cys) and 91 (Ala to Val) (Kim 2003, Maeda 2001, Cambau 1997, Matsuoka 2007, Matrat 2008, Rocha 2012). Several other mutations have been detected in countries such as Brazil, Colombia and India but remain to be validated: Ser92Ala, Ala91Thr, Leu97Phe, Arg107Leu (SBL, Suffys, Nora Cordona).

There is a need to update second-line drug treatment options for potential extremely drug-resistant leprosy cases (ie, dapsone, rifampicin and ofloxacin resistant). Newer drugs could be considered for evaluation, such as linezolid or those developed for tuberculosis including moxifloxacin, bedaquiline and delamanid.

The possible field causes of development of resistance are poorly understood. Some resistant cases received previous leprosy drugs; however, resistance is unlikely to develop during MB-MDT unless the strain was already dapsone resistant. If resistance is present for rifampicin and dapsone, clofazimine alone has low efficiency. Other sources of rifampicin exposure must also be considered, such as treatment for tuberculosis, prophylaxis or other infections. MB cases with BI of 2+ to 6+ (up to 10^{12} bacilli) harbour the greatest numerical risk of selection for resistance. PB cases with BI of 0 to 1+ (up to 10^{6} bacilli) are considered lower risk. In the French referral laboratory, a number of primary leprosy cases have been tested for resistance 2001–2012. None reported resistance for rifampicin (0/64), 15% for dapsone (8/55; P55L (5), T53I (2), T53A (1)), and 2% for ofloxacin (1/45 ; A91V). These primary resistant cases originated from New Caledonia (6), French Indies (1) and Mali (1). The one case resistant to ofloxacin had previously received treatment with ciprofloxacin for months due to prostatitis.

In summary, antibiotic resistance exists in leprosy and requires surveillance to ensure efficiency of MDT strategies as well as second-line drug options. Resistance can be due to previous leprosy therapy or from general use of antibiotics, which emphasizes the need to reduce general overuse of antibiotics. There is also a need to find new antibiotics for leprosy. A drug specific to leprosy, similar to dapsone and clofazimine, would be a better option than a broad-spectrum antibiotic, due to potential overuse of other antibiotics against other diseases common within those populations.

6.5 Inclusion of patients for drug resistance surveillance studies

Dr Paul Saunderson, Med. Director, American Leprosy Missions, said that a study was previously reported from the Philippines involving 500 BI+ MB cases treated between 1987–1994, with two years of MDT (Balagon, et al 2009). Cases experienced an average 12.8 years of follow-up. Twenty-three cases relapsed between 6 and 16 years RFT, relapse diagnosis peaking at 11–12 years. Cumulative risk for relapse was overall 6.6%, but showed a slightly lower risk of 5.0% for those with a pre-MDT BI < 4 and higher 10.1% risk for those with a BI 4+ or higher. All relapse cases were rifampicin sensitive by mouse foot-pad testing.

It was also noted that new leprosy cases within the USA do not receive standard MDT, but rather an alternative regimen administered through the National Hansen Disease Programme. PB patients receive two drugs daily, 100 mg dapsone with 600 mg rifampicin for one year; and MB patients receive three drugs daily, 100 mg dapsone with 600 mg rifampicin and 50 mg clofazimine for two years. Using this regimen has resulted in essentially no reported relapse cases from these patients (DL Williams; D Scollard; NHDP, Baton Rouge, USA).

The poor understanding of leprosy transmission combined with diagnostic challenges makes it difficult to determine if active disease after MDT is truly relapse. WHO defines relapse as new skin lesions arising after MDT completion, with an increase in BI of 2 or more at any site. Some suspect that many so-called relapse cases reported soon after the end of MDT, may actually be leprosy reactions unrelated to relapse (more common within 3-5 years RFT), or patients who are improving but have residual signs of disease, alongside a positive BI, which usually declines by 1 unit/year. In addition, some patients may not have actually received or taken adequate MDT, resulting in early disease recurrence (for example, MB cases misclassified and treated as PB; or non-compliance with prescribed treatment).

For drug resistance testing, relapse and other secondary cases are the most obvious priority, followed by primary cases, which are more likely to demonstrate resistance when secondary resistance is detectable in the population. If resistance is detected, new cases connected by geographical, genetic or social ties could be examined and potentially linked by epidemiological studies using WGS. Inactive cases or reactions should not be tested.

6.6 Drug resistance surveillance: conclusions from the preparatory meeting, Feb 4, 2014

Dr PV Ranganadha Rao, WHO-GLP reported that a preparatory meeting was organized on 4 February 2014, a day prior to DRS meeting to discuss the challenges faced in continuing surveillance and inclusion of surveillance network in national leprosy programmes. A small group consisting of national leprosy programme managers, experts in the field of leprosy on the DRS and clinical leprosy participated in the meeting. The following excerpts from the preparatory meeting were presented in the DRS meeting for further suggestions and comments.

- Because drug resistance in leprosy remains a concern, surveillance should be continued and expanded to maintain the integrity of MDT.
- Networks of sentinel centres should be developed for the Eastern African region covering Ethiopia, Tanzania and other endemic countries, similar to the Western African region.
- All countries reporting more than 1000 new cases annually need to develop a DRS programme with technical support from national referral centres.
- The network of sentinel surveillance centres need to include all national and sub-national level sentinel centres and reference laboratories.
- Patient data collection forms should include more clinical data and be converted into electronic format.
- Partnering laboratories need to ensure compliance to standardized guidelines in order to strengthen coordination between the laboratories and sentinel centres.
- Findings from the DRS programme need to be compiled and published for effective dissemination and wider distribution to health professionals, programme managers and policy-makers.
- Operationally, it is important to include surveillance of drug resistance alongside other leprosy control components within both global and national leprosy programme strategies.
- Advice should be sought from the WHO TAG on leprosy control on the definition, management and documentation of relapse cases as well as treatment regimens that could be better standardized and disseminated to clinicians, health workers and researchers.

7. Conclusions and recommendations

7.1 The definition of relapse

In general, participants are following the definition given in the WHO DRS guidelines (2009):

"A relapse is defined as the recurrence 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 bacteriological index of two or more units at any single site."

It is recommended that we continue using this consensual definition. Although there is usually a gap of some years between RFT and the diagnosis of relapse (especially an MB relapse), and this may be a useful pointer in distinguishing between a relapse and a reaction, the agreed definition does not include any reference to timing.

7.2 The management of cases with drug resistance

The treatment of proven rifampicin-resistant cases should follow the recommendations of the WHO Expert Committee 2010, which were confirmed in the report of the Cotonou meeting. Drugs will be provided to countries by partners for use after laboratory confirmation of resistance. A list of prequalified suppliers should be established by WHO.

7.3 The network of drug resistance surveillance (DRS) sites

Countries where more than 1000 new cases of leprosy are reported annually will be the focus for improved drug resistance surveillance. The network of sentinel centres should be further developed in East Africa, for example in Ethiopia, Tanzania and other endemic countries.

7.4 The laboratory network of the DRS

The current system of coordination between national programme and reference laboratories needs to be maintained. WGS seems to be a promising development which would allow a wider range of epidemiological data to be collected along with the drug resistance data; although still expensive and not yet widely available to the leprosy community.

7.5 Foci of resistant cases

A small number of foci of rifampicin resistance have been discovered, for example, in a former leprosy colony in Brazil and in West Bengal (India). It is recommended that particular efforts are made to investigate these situations, by testing as many new cases in the same area as possible.

7.6 Documentation of the drug resistance surveillance network

Publishing information on drug resistance and the network of sentinel centres is a high priority.

8. Closing

Dr Marivic Balagon**,** Executive Director, The Leonard Wood Memorial, Center for TB and Leprosy Research, Cebu, Philippines said that the past two days were very productive and informative.

Dr Sumana Barua, Team leader, WHO – GLP, said as per the presentations made over the last two days, drug resistance in leprosy is not a big problem at present but it is a potential future challenge. The GLP seeks to safeguard MDT for leprosy treatment in the future. An important challenge is to involve new laboratories in countries detecting more

than 1000 leprosy cases every year, so that all such programmes are represented in the surveillance network. Dr HJ Kawuma, Chairman, WHO TAG, thanked participants for raising good discussion points for consideration. He also thanked the local organizers for their support which has contributed to the success of the meeting. Dr Paul Saunderson, Medical Director, ALM thanked all the participants for meaningful discussions. Dr Ermesto ES Villalon III, National Programme Manager, NLCP, Phillipines. thanked WHO-GLP and ILEP for choosing Cebu as a venue for this important meeting and acknowledged that it had provided him with helpful insights into drug resistance in leprosy. Dr Christian Johnson, in his closing remarks said that the Fondation Raoul Follereau is glad to support this meeting with other ILEP members. He looked forward to strengthening of networking efforts.

Annex 1

Agenda

(1) Opening session:

(2) Updates on global leprosy situation, implementation of the Enhanced Global Strategy 2011–2015 and sentinel surveillance on drug resistance surveillance in leprosy

(3) Country presentations: Report on laboratory findings from relapse surveillance on mutations detected, trends and challenges

- Brazil
- China
- Colombia
- Benin, Guinea, Mali and Niger
- India
- Indonesia
- Madagascar
- Viet Nam
- Yemen
- Nepal
- The Philippines

(4) Drug resistance in leprosy patients: Diana R Williams, NHDP

(5) Whole genome sequencing (WGS) Discussion on further steps in Drug Resistance Surveillance- Professor Stewart Cole

(6) Review of past quality control studies in the DRS and criteria for inclusion of reference centre

(7) Management of drug-resistant cases

(8) Inclusion of patients for drug resistance surveillance studies

(9) Conclusions from preparatory meeting on drug resistance surveillance network

(10) Conclusions and recommendations

(11) Closing

Annex 2

List of participants

National Programme Managers

Guinea

Dr Fatoumata Sakho
Coordinator
National Programme of Fight Against Leprosy
Ministry of Health and Public Hygiene
Guinea

Dr Gouressi Sock
In charge of Monitoring and Evaluation National Programme of Fight Against Leprosy
Ministry of Health and Public Hygiene
Guinea

Madagascar

Dr Randrianantoandro Andriamira
Chief of the Leprosy Programme of Madagascar

Dr Abdul Rahim Al-Samie
Director
National Leprosy Elimination Programme
Skin and Venereal Diseases Hospital
Taiz, Yemen

Mozambique

Dr Olga Amiel
Ministry of Health
Leprosy Programme Manager
Mozambique

Nepal

Mr Chuda Mani Bhandari
Director Programme Manager
Leprosy Control Division
Department of Health

People's Republic of China

Dr Shen Jianping
Deputy Director, Department of Leprosy Control
Institute of Dermatology and National Center for Leprosy Control
P.R. China

The Philippines

Dr. Ermesto ES Villalon III
National Programme Manager
National Center for Disease Prevention & Control
Department of Health
Manila

Dr Francesca Gajete
Former National Programme Manager,
National Center for Disease Prevention and Control,
Department of Health, Manila

Focal points from sentinel sites

Ms Elaine Faria Morelo
Ministry of Health Surveillance
Secretariat on Communicable diseases Surveillance
Commercial Sector Elimination South
Brasília DF
Brazil

Dr Patricia Sammarco Rosa
Instituto Lauro De Souza Lima
Bauru-Sao Paulo
Brazil

Dr Philip Suffys
Head of the Laboratory of Molecular Biology Applied to Mycobacteria
IOC – Fiocruz
Rio de Janeiro
Brazil

Dr Juan Camilo Beltrán Alzate
Instituto Colombiano de Medicina Tropical - Universidad CES
Colombia

Dr Jonnalagada Subbanna
Director, LEPRA Society
Blue Peter Public Health & Research Centre (BPHRC)
Cherlapally
Hyderabad 501 301, Andhra Pradesh

Dr Mallika Lavania
Research Scientist
Stanley Browne Laboratory
The Leprosy Mission Community Hospital
Delhi, India

Mr Sundeep Chaitanya
Research Officer
Stanley Browne Laboratory
The Leprosy Mission Trust India
TLM Community Hospital
Nand Nagri, Shahdara
Delhi-110093

Dr Deanne Hagge,
Head of Laboratory,
Anandban, Hospital,
Kathmandu
Nepal

Dr Mahesh Shah
Anandban Hospital,
Kathmandu
Nepal

Professor Wang Hongsheng
Associate Professor
Institute of Dermatology
Chinese Academy of Medical Sciences & Peking Union Medical College
National Center for STD and Leprosy Control, China CDC.
China

Dr Marivic F Balagon
Executive Director
Leonard Wood Memorial
Cebu
Philippines

Dr Armi A. Maghanoy
Acting Chief
Cebu Skin Clinic
Cebu, Philippines

Dr Diana L. Williams
Molecular Biology Research Section
DHHS, HRSA, HSB
Division
National Hansen's Disease Programme
Laboratory Research Branch
LA, USA 70803

Collaborating reference laboratories

Professor Emmanuelle Cambau
Professeur de Bactériologie-Université
Paris Biderot
Centre de Référence Mycobacteries

Dr Masanori Kai
Researcher
Leprosy Research Center
National Institute of Infectious Diseases
Tokyo, Japan

Professor Stewart Cole
Professor
EPFL / GHI
Lausanne,
Switzerland

Dr Masanori Matsuoka
Researcher
Leprosy Research Center
National Institute of Infectious Diseases
Tokyo, Japan

Temporary advisers/Experts

Dr C M Agrawal
Deputy Director General (Leprosy)
National Leprosy Eradicaiton Programme,
New Delhi
India

Dr Herman Joseph S. Kawuma
Chairman, WHO Technical Advisory Group for Leprosy
Medical Adviser
German Leprosy Relief Association (GLRA)
Kampala
Uganda

Dr Rosa Castália França Ribeiro Soares
Coordinator
General Coordination of Leprosy and diseases targeted for elimination
Brasilia, DF, Brazil

Dr Christina Widaningrum
Head, Subdirectorate of Leprosy and Frambusia
Directorate of Communicable Diseases
Directorate General of Control Diseases and Environmental Health,
Ministry of Health
Republic of Indonesia

Experts from partner agencies

American Leprosy Missions

Dr Paul Saunderson
Medical Director
American Leprosy Mission
Cebu, Philippines

Raoul Follereau Foundation

Dr Roch Christian Johnson
Médecin
Raoul Follereau Foundation

Dr Bertrand Cauchoix
Clinical Adviser
Raoul Follereau Foundation

WHO Secretariat

Dr Katsunori Osuga,
Acting Team Leader--STB and Leprosy Elimination
WPRO WHO Manila

Dr Woojin Lew
Medical Officer for STB and Leprosy Elimination

Dr Maria Gemma Cabanos
Focal Person for leprosy

Regional Office for South-East Asia, New Delhi

Dr Sumana Barua
Team leader,
Global leprosy programme, WHO

Dr V R Rao Pemmaraju
TIP GLP,
WHO New Delhi, India

Annex 3

Reference laboratories collaborating with the WHO Global Leprosy Programme for sentinel surveillance of rifampicin resistance

(1) Department of Microbiology
Yonsei University College of Medicine
134 Shinchon-dong, Seoul 120-752, Republic of Korea
Tel. No.: +822-2228-1819; Fax No.: +822-392-9310
Email: raycho@yonsei.ac.kr

(2) Leprosy Research Centre
National Institute of Infectious Diseases
4-2-1 Aoba-cho, Higashimu-rayama-shi
Tokyo 189-0002, Japan
Tel. No.: 81-42-391-8211, Fax No.: 81-42-394-9092
Email: mkai@nih.go.jp

(3) Central JALMA Institute for Leprosy and Other Mycobacterial Diseases
Dr M. Miyazaki Marg, Taj Ganj
Agra – 282 001, India
Tel. No.: +91-562-2331751, Fax No.: +91-562-2331755
Email: vishwamohan_katoch@yahoo.co.in

(4) Global Health Institute
Ecole Polytechnique Federal de Lausanne
EPFL SV/GHI/UPCOL,
Station No.19, CH-1015 Lausanne, Switzerland
Tel. No. +41 21 693 1851, Fax No. +41 21 693 1790
Email: stewart.cole@apfl.ch

(5) Oswaldo Cruz Foundation
Ministry of Health, Av. Brazil 4365
Manguinhos Pavilhao Mourisco S. 105
21045-900 Rio de Janeiro, Brazil
Tel. 55 21 2590 4712, Fax: 55 21 2590 9741
Email: euzenir@uol.co.br

(6) Division of National Hansen's Disease Programme
Laboratory Research Branch
Louisiana State University, School of Veterinary Medicine
Room 3517W, Skip Bertman Drive, Baton Rouge, LA 70803
United States of America
Tel: 225-578-9836, Fax: 225-578-9856; Email: tgillis@lsu.edu

(7) National Reference Center for Mycobacteria and resistance to anti-tuberculosis drugs
Department of Bacteriology and Hygiene
Faculty of Medicine
91 Boulevard de Hospital
75634 Paris Cedex 13, France
Email: Emmanuelle.cambau@lrb.aphp.fr

(8) LEPRA - Blue Peter Public Health and Research Center
Immunology and Molecular Biology Division, Near TEC Building
Cherlapally, Hyderabad 501 301
Andhra Pradesh, India
Tel: 40 272 64547
Email: subbanna@leprahealthinaction.in

(9) Stanley Browne Laboratory
The Leprosy Mission Community Hospital
Nandnagri, Delhi 110093, India
Tel: 91 11 225 94295
Email: mallikalavania@gmail.com

(10) Department of Microbiology, Immunology and Pathology
Colorado State University
Fort Collins CO 80523
United States of America
Tel: 970 491 0751/4139/6390, Fax: 970 491 1815
Email: weili@colostate.edu

Anti-microbial resistance is an important factor to reckon in communicable disease control and leprosy is no exception. Rifampicin is a strong constituent antibiotic in the multidrug therapy (MDT) besides dapsone and clofazimine. Even after 30 years since its introduction, MDT remains the only WHO recommended regimen for treating leprosy. Surveillance of drug resistance is a necessary particularly in the absence of new drug regimens as effective as MDT.

In 2009, WHO published 'Guidelines for Global Surveillance of Drug Resistance in Leprosy'. Currently drug resistance surveillance (DRS) is carried out by screening all multi-bacillary (MB) patients who have relapsed after completing the prescribed WHO MB-MDT, on a sentinel centre basis.

Currently 17 countries are participating in surveillance activities by collecting patient samples for testing drug resistance: Benin, Burkina Faso, Brazil, Colombia, China, India Indonesia, Madagascar, Mali, Mozambique, Myanmar, Nepal, Niger, Pakistan, Philippines, Viet Nam and Yemen. There are ten reference laboratories which facilitate processing of these samples for 'leprosy drug resistance DNA mutation' detection in Brazil, France, India, Japan, Nepal, South Korea, Switzerland and USA. Because drug resistance in leprosy remains a concern, surveillance should be continued and expanded to maintain the integrity of MDT. Networks of sentinel centres should be developed for the eastern African region covering Tanzania, Ethiopia and other endemic countries, similar to the western African region.

The meeting on drug resistance surveillance was organized to review the progress on drug resistance surveillance in leprosy through the network of sentinel centre. Currently rifampicin resistance does not seem to be a serious problem among relapse cases. Longitudinal observation need to be continued since the situation in leprosy control is not the same situation as in TB or other disease control programmes and vigilance has to be maintained to prevent the occurrence and spread of drug resistance and thus maintain the effectiveness of MDT.

World Health Organization
Regional Office for South-East Asia
World Health House
Indraprastha Estate
Mahatma Gandhi Marg
New Delhi-110002, India

SEA-GLP-2014.1