Sentinel surveillance for drug resistance in leprosy

Report of the WHO Global Leprosy Programme Meeting
Cotonou, Benin, 12–13 November 2012

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Acronyms

BI	Bacterial Index
BPHRC	Blue Peter Public Health and Research Centre
DDS	Diamino-diphenyl sulfone
DNA	Deoxyribonucleic acid
DRDR	Drug resistant determining regions
DRS	Drug resistance surveillanceiv
FRF	Raoul Follereau Foundation of France
GLP	Global Leprosy Programme
HRM	High Resolution Melt Analysis
ILEP	International Federation of Anti-Leprosy Associations
ILSL	Instituto Lauro de Souza Lima
MB	Multibacillary
MDT	Multidrug therapy
MFP	Mouse footpad
NCDR	New Case Detection Rate
NLEP-HQ	National Leprosy Elimination Programme Headquarters
PCR	Polymerase chain reaction
QC	Quality Control
SBL	Stanley Browne Laboratory
SINAN	National Information System for Notifiable Diseases
SSS	Second skin smear
SVDH	Skin and Venereal Diseases Hospital
TLM	The Leprosy Mission

1. Background

Multidrug therapy (MDT) has been successfully used to treat leprosy throughout the world for three decades, and rifampicin remains the key component. It is known that resistance to rifampicin can occur relatively easily if it is used as monotherapy, but global surveillance for resistance has not been feasible until the recent development of suitable molecular methods. Now that these methods are available, it is important that levels of resistance are monitored. A network of sentinel sites was established in 2007, with annual meetings to share data and technical developments, and to make recommendations. The annual meeting also monitors trends in development of new drugs of relevance to leprosy control.

2. Objectives

The objectives of the meeting were to:

(1) review the drug resistance surveillance data and other relevant issues in specimen collection, analysis and reporting;

(2) review trends in relapses reported by national programmes;

(3) review and update recent advances in deoxyribonucleic acid (DNA) sequencing technology.

3. Opening session

Professor Stewart Cole of the Global Health Institute, Lausanne, Switzerland and also consultant to the Raoul Follereau Foundation of France (FRF), welcomed participants and thanked the FRF for handling the local arrangements. The FRF supports several programmes in Africa and it was good to see greater involvement of African participants at the meeting.

Dr Raoul S Chabi of WHO-Benin welcomed the meeting participants to Benin, noting the success of the treatment of leprosy in recent years, with the donation of free medicines, initially by The Nippon Foundation, and subsequently by Novartis.

Dr Loko Frederick, representing the Minister of Health, Benin, also welcomed participants to Cotonou, and commented that while leprosy was no longer a major problem, new cases of leprosy were still occurring and residual morbidity was still an issue for health services to deal with.

4. Update on the global situation

Dr Vijay Pannikar reviewed the leprosy situation, pointing out that globally the number of new cases had been static for the past five years. Three countries, Brazil, India and Indonesia represented 85% of the global burden. He listed a number of challenges being faced by public health services:

- accessibility to treatment in remote areas
- provision of referral services
- supportive monitoring and supervision
- information, education and communication (IEC), capacity building and partnership
- prevention of drug resistance
- new measures to further reduce the incidence of leprosy, including chemoprophylaxis and immune-prophylaxis.

Within the sentinel surveillance network, 17 countries and ten reference laboratories participate. Since 2004, the number of relapse cases reported to WHO has been quite stable (from 2000 to 3000 cases annually), with Brazil, China, India, Indonesia and Nigeria reporting the highest numbers. Dr Pannikar said that it was necessary to test a proportion of new (never-treated cases) for drug resistance, so that a larger number of samples could be tested each year, in total.

Although there was an agreed drug regimen for resistant cases, it had not been tested in a clinical trial, which was not an ideal situation. Dr Pannikar reminded participants that when dapsone (DDS) resistance became a serious concern for the treatment of leprosy, MDT, which was in use, was developed and implemented in a state of emergency after a few clinical trials. Fortunately, rifampicin had been a miracle drug and relapse rates had been low. However, he emphasized that it took years to select, test and conduct clinical trials for new drugs, because "relapse" was the measured outcome, which in the case of leprosy could be years or even decades after treatment completion. "Therefore, laboratory and clinical trials should be in place today", he said. Also, ofloxacin resistance may be on the increase, which could pose a problem for newer antileprosy regimens.

During the meeting, Dr Pannikar repeatedly cautioned that the clinical definition of relapse was not clear in the MDT era. The regimens had changed from dapsone monotherapy for life, to WHO MDT till smear negativity, to fixed-duration, two-year MDT, and most recently to a one-year MDT regimen. So, how soon after treatment and with what criteria (clinical or laboratory) a patient presented as "relapse" was an undefined and evolving issue for clinicians. Therefore, there was a need to compile a large amount of data on relapses after MDT, from different centres.

The participants were informed that WHO was planning to establish a new subgroup on chemotherapy, and that it was grateful to ILEP for providing the resources for its meeting.

5. Relapse cases

5.1 Country presentations

(1) Presentations on **Mali** and **Benin** were made jointly by Dr Kodio Mamoudou and Dr Fomba Abdoulaye. Mali reported 363 new cases in 2011 (NCDR 4.7/100 000) and Benin 246 (NCDR 2.2/100 000). The molecular DRS was applied for 34 cases in Mali and for 4 cases in Benin, and tested for drug resistance in Paris (France) and Lausanne (Switzerland). The results for 7 MB relapse cases (all in Mali) and 31 new cases (27 in Mali and 4 in Benin) were as follows:

29 wild type strains, and 2 cases tested for drug resistance in Lausanne, Switzerland. Two cases of dapsone resistance were identified among the new cases (one in Benin and one in Mali). Rifampicin or ofloxacin resistance was not detected in the relapses or new cases. Genotypes were also examined, most being the 4N subtype and a few of the 4O subtype, although interestingly, some with the 4P subtype which were until now regarded as having evolved in South America were detected; i.e. this subtype strain is also of African origin.

(2) Regarding **Yemen,** Dr Abdul Rahim Al-Samie said that difficulties had been encountered in recent years because of the widespread sense of insecurity following the demolition of health clinics during the youth revolution. However, treatment of cases was being carried out effectively. There were 299 new cases in 2011 (NCDR 1.3/100 000) with 13% being under 15 years, 53% classified as MB and 9% presenting with grade-2 disabilities. Three relapse cases were suspected and diagnosed at the peripheral clinics, but patient referrals to the National Leprosy Referral Centre (NLEP- HQ), the Skin and Venereal Diseases Hospital (SVDH) for sample collection for quality control (QC) of BI and molecular tests, were yet to be completed. Meanwhile, the patients had begun retreatment.

(3) Data for **Nepal** were presented by Dr Chuda Mani Bhandari. He spoke in detail about the national leprosy indicators, the infrastructure and the national 2011–2015 strategy for leprosy. In 2011 (NCDR 12.2/100 000), 3481 new cases were registered, with 83% cases being in the Terai area (the plains bordering India). With regard to the relapse confirmations and drug resistance surveillance by molecular methods, clearance from the government was obtained in 2010, with the arrangement that the referral and laboratory duties would be assigned to the Anandaban Hospital near Kathmandu. Prior mouse footpad-based DRS (from 2000–2010) suggested 75 DDS resistance cases

(mostly low-level resistance) and 4 with rifampicin resistance (2 being DDS and Rif multidrug resistant). A total of 24 relapses since 2011 (20 at Anandaban, and 2 each from Lalgadh and Green Pastures clinics) were thought to be pending investigation.

(4) With regard to **Madagascar**, Dr Andriamira Randrianantoandro explained that there was a stable detection of about 1500 cases per year, with 12% being under 15 years of age and 84% being classified as MB. The grade-2 disability rate was 22%, which reflected the difficulty in providing services in remote areas. Starting in 2010, with the intention of setting up drug resistance surveillance and joining the global network, Madagascar used the criteria of laboratory capacity to identify six sentinel sites as of April 2012. Laboratory work was to be partnered with Centre de Référence Mycobacteries, Paris. Since 2010, a total of 38 relapse cases had been diagnosed, of which only 7 had been biopsied for molecular tests (pending). Seven new cases and seven relapse cases had been sampled with 10 wild type strains on drug resistance results and four negative polymerase chain reactions (PCRs) (two new cases on treatment and two relapse cases).

An issue raised in the discussion was whether a biopsy was needed for the laboratory work, or would a skin smear do. It seems that if the smears are well taken, they are adequate, but not all staff members have had sufficient training, so a punch biopsy may be better. This is an interesting paradox, since an increase in BI of ≥ 2 is an optional criterion in relapse diagnosis. In India, the material remaining from the blade and transferred to ethanol after smearing on glass slides for BI measurements is of variable quality and quantity. It has been possible to obtain good PCR results from the stained smear (i.e. from material on the glass slide). There was discussion about the ethical requirements for requesting a second skin smear (SSS) and the implication of the time lapsed after the patient would have been placed on re-treatment (resulting in decrease in BI and thus DNA). Thus the timescale to report results from molecular laboratories to clinics should be discussed in more detail.

The high disability grade level was a matter of concern. Dr Randrianantoandro stated that passive case-finding was insufficient in Madagascar. However, it was clear that the country was facing poverty and resource limitations. He described the circumstances causing a paucity of available supplies (such as punch biopsy kits), infrastructure, training and good transport.

5.2 Technical discussions

(1) **Clinical implementation of next generation sequencing: application to leprosy**

Professor Stewart Cole reviewed the contribution of microbiology to clinical medicine under three headings: diagnostics; resistance profiling; and surveillance. For most organisms, standard methods based on culture have

become both automated and sophisticated, but as the leprosy *bacillus* cannot be grown in a laboratory, molecular methods are better. The DNA sequencing can give reliable results, very rapidly, in a cost-effective manner. Resolution is down to the level of a single nucleotide. The various high-end and smaller personal sequencing machines currently available were reviewed, and some of the practical problems (such as methods of enriching the specimens, separating the *M. leprae* DNA from host DNA) were analysed. Professor Cole described two methods – the first based on an *M. leprae* oligonucleotide capture array and the second based on anti-PGL-1 antibodies to capture unbroken bacteria. The former is more expensive and arrays have to be re-used to reduce costs. The latter has been useful for even small amounts of tissue (such as biopsies). Ten strains of *M. leprae* have now had their whole genome sequenced, and this is becoming possible on smaller and smaller samples – it should soon be possible to sequence the genome directly from a biopsy, without the need to grow the organism in the mouse footpad (MFP) first. This will allow closer surveillance of transmission patterns, mixed and coinfections, and any other genetic variation associated with the development of drug resistance.

In discussion, the question of persisters was raised, but Professor Cole indicated that there was no evidence yet that persisters were genetically different in any way. He also said that as new antibiotics were developed for tuberculosis, one could usually predict their likely value in leprosy and identify resistance patterns as they developed; at present, clofazimine remains an enigma, as we do not know its mechanism of action.

(2) **Sensitivity and specificity issues in molecular and mouse footpad growth assays for drug resistance surveillance of clinical isolates**

Dr Varalakshmi Vissa presented her analysis of real-time PCR-based high resolution melting (HRM) curves that can be obtained directly from the PCR machine, after the completion of amplification without any further operator-dependent sample handling. There is no requirement for any mutation-specific primers or probes, besides conventional PCR primers. If there is a mutation, the melting curve will be distorted, so this could be a simple screening tool: only samples suggestive of a mutation would be sequenced (Li et al., 2012, JCM). The HRM method was applied to 218 samples drawn from a 10-year repository (2000–2010) from Anandaban Hospital, 6 of which were suspected relapses. The relapse samples do not have DRDR mutations in *folP1* and *rpoB* (by the HRM analysis and DNA sequencing). A large number of the *folP1* amplicons have also been sequenced; thus far, one mutant has been identified that correlates with an HRM variant curve. The *rpoB* locus was not examined by an HRM or sequencing, except for the 6 suspected relapses and 18 other samples. Of these, two with HRM variant curves had mixed alleles (wildtype and mutant bases at codon 456) in the amplicon sequence suggestive of hetero-resistance.

A separate, smaller batch of coded samples including aliquots of the primary biopsy and mouse footpad-derived homogenates, related to the 218 cases,

was re-examined by PCR and sequencing, because some of these were shown to be resistant to DDS or rifampicin or both in MFP assays. Two samples were from patients also infected with TB. However, preliminary results do not show any correlation between the MFP and sequencing results. i.e. mutations in DRDR were not detected in either the primary homogenate or the paired mouse footpad-derived sample. The number of MFPs with growth in the presence of drug varied; not all MFPs inoculated (2 per animal, at least 5 animals per drug group) were positive for growth in the animals that survived the 6-9 month experimental duration.

Further molecular tests are necessary to re-test some of the suspect DR samples, to fill gap for any PCR and/or sequence failures, and to complete the analyses of all 218 samples for *rpoB* and *folP1* targets.

6. Laboratory findings

6.1 Country presentations

(1) The data from **Hyderabad, India**, were presented by Dr Subbanna from the LEPRA Blue Peter Public Health and Research Centre Laboratory (BPHRC). Samples come from Andhra Pradesh and Orissa, and the sequencing is outsourced locally. 29 relapse cases were investigated, but only 11 were PCR positive in 2011. Of those that were PCR and sequence positive, DRDR mutations were not detected. 131 suspected relapses and 54 new cases have been entered into the surveillance study. Of those with DNA results, three *folP1* mutant cases have been detected – two in relapse cases and one in a new case. The clinical histories of 13 of 29 relapse cases reported in 2011 were presented. In two cases the primary episode had occurred as recently as 2010.

(2) Dr Paul Saunderson presented data from the **Philippines**; nine relapse cases were identified in Cebu in 2011 and biopsies were sent to Dr Diana Williams at the National Hansen's Disease Programmes in Baton Rouge, United States of America. All nine specimens showed sensitivity to all three drugs, DDS, rifampicin and ofloxacin, based on the multiplex PCR-sequence approach. The time lapse between the first (one or two-year MDT) and relapse diagnoses (all of LL type) ranged from 6 to 24 years.

(3) Dr Nora Cardona-Castro presented data from **Colombia**. The country normally witnesses 20–25 relapse cases every year. Dr Castro and her colleagues attempted to review 69 relapses reported during 2010–2011, to examine the clinical details, assess BI, and conduct molecular tests: 38 cases could be traced, 24 were BI positive, and only 7 cases were confirmed as relapses, while other diagnoses included reactions, persistent high BI after treatment, non-compliance, misdiagnosis due to misinterpretation of the WHO relapse definition and other medical complications of leprosy. Of the seven confirmed

relapses, one case had a mutation in the *rpoB* gene, but not the one associated with resistance.

The definition of relapse was discussed. Professor Cambau suggested that the cases could simply be divided into untreated cases and previously-treated cases; this would certainly be practical when looking for drug resistance. Dr Saunderson pointed out during discussions that this would not help clinicians who need to decide if the person requires further treatment. Dr Pannikar suggested that the current definition of relapse be retained, but that individual cases be looked into more carefully. There are still no means of easily distinguishing relapse from re-infection and many health workers have difficulty distinguishing between relapse and reactions in patients who have completed treatment.

(4) Dr Mallika Lavania presented data from **Delhi, India**. The Stanley Browne Laboratory (SBL) receives samples from 19 The Leprosy Mission hospitals across India, but most come from three large centres. Each year 30–50 relapse cases are usually reported (52 cases in 2011, 34 tested at SBL) and the DNA sequencing is outsourced locally. Of the 34 samples tested, there were 5 cases of DDS resistance, 5 cases of ofloxacin resistance, but zero rifampicin resistance. Including the two cases with *gyrA* mutations detected in 2009, the total of seven such cases is a concern. One of these cases is a relapse case occurring after 36 years, and has *folP1* and *gyrA* mutations.

Concern was expressed regarding the level of ofloxacin resistance. This is presumably due to the use of the drug for other incidental infections, as it is not being used for leprosy. This resistance would affect all quinolones, so this finding casts doubt on the future usefulness of drugs such as moxifloxacin, which has been seen as the best reserve drug for leprosy. It is to be noted that 5 have a Ser 92 mutation, a type that has not been reported for *M. leprae* and thus not included in the two current array-based detection systems. It would be interesting to identify which specific drug is causing this particular mutation type in Indian patients.

(5) Dr Kai presented data from **Myanmar**. New cases are fairly static at around 3000 new cases per year, with 18 relapses reported in 2011. Fifteen samples were tested in Japan, with only one case of DDS resistance.

(6) There were two presentations from **Brazil**. There are 4 designated regional sentinel referral centres in Brazil, each supporting 5–10 states.

Dr Philip Suffys from the Fiocruz Centre, presented results from 145 patients from five states (Phase 1: 2006–2009, published Adalgiza da Silva Rocha, JCM, 2012), and another 188 patients (Phase 2: 2010-2012). It appears that of the total relapses officially reported for the whole country in the national notifiable disease information system (SINAN) during 2010-2012, only 10% have been directed to the four sentinel centres for molecular tests. Fiocruz has received cases from 3 of the 10 states that come under its coverage Rio De Janerio, Espirito Sante and Districo Federal. From the PCR and sequence-positive data

from the 333 cases that cover the two phases, 5 cases had rifampicin resistance (one with heteroresistance), 3 had *folP1* mutations and 2 had *gyrA* mutations (besides part of the population showing an SNP not related to DR in codon 91). Only two cases were found to be resistant to all the three drugs tested. Patients with rifampicin resistance had a shorter time to relapse than those without (3.3 years versus 10.2 years); sequencing of part of the samples from 2012 is under way.

Dr Suffys also reviewed published genotyping results for eight relapse cases, five of whom were re-infected with a different strain (Adalgiza da Silva Rocha Journal of Medical Microbiology, 2011). He presented results of other ongoing trials with different specimen types (second skin smear versus biopsies), and examples of mixed or persistent infections. He also showed how the Biomerieux/Hain Genotype LepraeDR array gave results as expected when tested with the sequenced Phase 1 samples. The array was not sensitive for PB samples.

Dr Patricia Rosa, representing the ILSL sentinel centre, presented DRS results of 161 cases (study period 2008–2012) from within the institute (n=78), referred (n=59) and those from a survey of treated cases and contacts in a former leprosy colony (Santo Antônio do Plata, in Pará State, n=24). In the PCR positive group, they detected 5, 2 and 0 cases with mutations in *folP1*, *rpoB* and *gyrA* respectively. In addition, 11 had double mutations in *rpoB* and *folP1*. In the Prata colony, they examined 207 residents and found 12 relapse cases among 104 former patients, and 10 new cases among the 103 household contacts examined. Of the 12 relapse cases identified from the former patient group, 6 had rifampicin and DDS dual resistance. Out of the 10 new cases, 3 had rifampicin resistance, 2 of whom were also DDS resistant. In all, three cases (from both the groups) with single resistance to DDS were seen. The VNTR typing and family links showed an indication of transmission among residents.

In discussion, Dr Saunderson asked about the treatment and follow-up of these drug-resistant cases in Pará. Dr Rosa said that they would be reviewed next year by ILSL. In the meantime, they are using the recommended alternative drug regimen. Dr Pannikar mentioned that in the early days of MDT there was some resistance to using clofazimine, either because of cost, or because of its side-effects. Since 1996, all countries are using the recommended regimen. Professor Cole pointed out that ofloxacin resistance seemed to be lower in Brazil than in India.

(7) Dr Nguyen presented data from **Viet Nam**, where 374 new cases and 5 relapses were diagnosed in 2011. Molecular results from the relapses are pending.

6.2 Further technical discussions

(1) **The results of the fourth quality control study**

Dr Masanori Matsuoka presented his review in which test samples were sent from Japan to reference laboratories for analysis. Twelve out of 17 reference laboratories reported results, and only 1 was somewhat below par. Dr Vissa suggested that QC of laboratory processes based on MFP-enriched bacilli is necessary. As more laboratories are joining the surveillance programme, it is time that QC is extended to cover clinical samples. It is important to study the clinical samples and PCR results together in greater detail considering various factors like clinical and bacteriological status. Clinical samples with PCR negative results should be studied in greater detail, with regard to sample (whether BI negative, positive or from a misdiagnosis, quantity and quality, PCR conditions etc.) Primer re-design or nested PCR, or other tests for *M. leprae* detection (such as 16S rRNA or RLEP) have not yet been formally instituted.

Professor Cole concurred with the idea and suggested that QC methods need to be considered for clinical samples and routine internal quality control methods should be continued for laboratory tests.

Therefore, it is suggested that individual laboratories should develop internal and external sample testing within the network. It is not feasible for Dr Matsuoka to collect and redistribute clinical samples for QC.

Dr Matsuoka asked whether sequencing of the *gyrB* gene should be added or not, since quinolone resistance caused by the mutation in the *gyrB* gene had been reported. Professor Cambau suggested that only samples that showed rifampicin resistance be analysed.

(2) **Update on new antimycobacterial drugs and their possible application for leprosy chemotherapy**

Professor Cambau discussed briefly the second-line drugs that are currently available, in particular, ofloxacin, minocycline and clarithromycin, although nothing is known about resistance to the latter two drugs. The other older drugs of interest include rifapentine, which has a longer half-life than rifampicin, but which would suffer cross-resistance with it; and moxifloxacin, which is as potent as rifampicin, but would suffer cross-resistance with ofloxacin.

The most promising new drug is the diarylquinolone, TMC-207, also known as bedaquiline, which is comparable to rifampicin in potency, has an entirely new mechanism of action and a long half-life. Some new anti-TB drugs are known not to be of use in leprosy (e.g. linezolid and PA-824), while others have not yet been tested e.g. delamanid and the benzothiazinones.

(3) **Clinical aspects of drug-resistant cases and experience in their management**

Dr Saunderson presented the clinical features of DDS resistance, including the appearance of histoid nodules, but noted that this was only of historical interest, as drug-resistant cases no longer showed any special clinical features. The vast majority of relapse cases will not contain resistant organisms and will respond well to an additional course of standard WHO-recommended MDT. DDS resistance is not clinically significant and does not require any change of regimen. If rifampicin resistance is proven, the regimen recommended by the WHO Expert Committee on Leprosy, Eighth Report, 2010 (WHO Technical Report Series 968) should be used:

- **Daily treatment** for 6 months, comprising:
  - Clofazimine 50 mg and any **two of the following:**
    - Ofloxacin 400 mg
    - Minocycline 100 mg
    - Clarithromycin 500 mg
- Followed by **daily treatment** for 18 months, with:
  - Clofazimine 50 mg
  - Either Ofloxacin 400 mg or Minocycline 100 mg

7. Conclusions and recommendations

The following were the conclusions and recommendations:

(1) Participants appreciated the arrangements made by FRF in Cotonou, Benin. The meeting was organized by the WHO Global Leprosy Programme (GLP), and funded by ILEP members, in particular, the FRF and the American Leprosy Missions. The presentation of results from a number of African and Middle Eastern countries (Benin, Madagascar, Mali, Nigeria and Yemen) was a welcome development at this meeting. Colleagues from China were unable to attend due to visa considerations.

(2) Up-to-date relapse case-finding and drug resistance results were presented from a number of countries [Africa (Benin, Madagascar, Mali, Niger, Yemen), Asia (India, Myanmar, Nepal, Philippines) and South America (Colombia, Brazil)]; it is gratifying to see that rifampicin resistance remains rare in all countries reporting on 2011–2012 cases, except in Brazil (particularly in former leprosy colonies), so that standard WHO-recommended MDT regimens remain the treatment of choice for leprosy everywhere. Dr Pannikar suggested that in countries where clofazimine has been routinely used from the start of the MDT era (as recommended by WHO), rifampicin resistance is hardly seen at all.

(3) Quinolone resistance is a concern, arising as ofloxacin is widely used for ordinary infections; this trend suggests that quinolones may not be a suitable

component of future standardized antileprosy regimens. This was strikingly more frequent in the cases coming to TLM and SBL, India. Whether the presence of *gyrA* mutations is more frequent in relapse cases or in the general population is not known, and surveillance in new cases should be included. Studies on the efficacy of alternatives drugs are, therefore, important.

(4) The definition of relapse remains as before (see Guidelines for Global Surveillance of Drug Resistance in Leprosy, WHO, 2009). Colombia did an elegant study to verify the officially reported relapse cases and found a serious over-diagnosis. Not all centres described what patient management procedures were; for example, were all "relapses" being retreated upon admission, independent of the results from the molecular tests? The guidelines for reporting results and the relationship between the clinic and the molecular surveillance sites with regard to patient care were not adequately and systematically reviewed. As this programme is developing in scale and scope, this issue should be addressed. Many patients that come under the surveillance programme have PCR-negative results. The implications of this group of cases need to be addressed in more detail (misdiagnosis, PB relapses and poor sample quality) to enhance the amount of data collected from clinical surveillance.

(5) The treatment of proven rifampicin-resistant cases should follow the recommendations of the WHO Expert Committee on Leprosy, Eighth report, 2010 (WHO Technical Report Series 968).

(6) Several countries presented more in-depth clinical information about relapse cases; this should be expanded so that the present-day profile of relapse cases can be more clearly defined and published. Follow-up information about the management of relapse cases should be collected and reported.

(7) Technical problems in determining drug resistance patterns have largely been resolved, and new methods are being developed; it is recommended that surveillance is expanded, both in terms of sentinel sites, collaborating laboratories and the cases to be tested; any previously treated leprosy cases with unexpectedly active disease, can be tested, whether or not they meet the strict definition of relapse. A sample of new cases can be included, up to the limit agreed to with the collaborating laboratory. Coinfections and hetero-resistance are being shown by more than one investigator/study site.

(8) The QC programme managed by the National Institute of Infectious Diseases, Tokyo, Japan, has helped to maintain reliability and should be modified to focus on clinical samples through internal and external controls.

(9) The WHO-initiated surveillance network, with its annual meetings, is a valuable forum for both scientists and programme managers, and should be continued. In this regard, there were several requests from presenters for financial support to compensate, at the minimum, the laboratory and sequencing costs.

(10) The next meeting of the network is proposed again on 19-20 November 2013, in Cebu, Philippines.

Annex 1

Agenda

- Opening session
- Updates on global leprosy situation and implementation of the Enhanced Global Strategy 2011–2015
- Country presentations on relapses: numbers, trends and management policies
- New development in mycobacterial genomics
- Next-generation approaches to culture *M. leprae*
- Sensitivity and specificity issues in molecular and mouse footpad growth assays for drug-resistance surveillance of clinical isolates
- Results of the fourth quality control study
- Update on new antimycobacterial drugs and their possible application for leprosy chemotherapy
- Clinical aspects of drug-resistant cases and experiences in their management
- Conclusion and recommendations

Annex 2

List of participants

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Annex 3

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

(1) Department of Microbiology
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Seoul, Republic of Korea

(2) Leprosy Research Centre
National Institute of Infectious Diseases
Tokyo, Japan

(3) Central JALMA Institute for Leprosy and Other Mycobacterial Diseases
Dr M. Miyazaki Marg, Taj Ganj
Agra, India

(4) Global Health Institute
Ecole Polytechnique Federal de Lausanne
EPFL SV/GHI/UPCOL
Lausanne, Switzerland

(5) Oswaldo Cruz Foundation
Ministry of Health, Av. Brazil
Manguinhos Pavilhao Mourisco S. 105
Rio de Janeiro, Brazil

(6) Division of National Hansen's Disease Programme
Laboratory Research Branch
Louisiana State University, School of Veterinary Medicine
LA, United States of America

(7) National Reference Centre for Mycobacteria and resistance
to anti-tuberculosis drugs
Department of Bacteriology and Hygiene
Faculty of Medicine
Paris, France

(8) Immunology and Molecular Biology Division
Blue Peter Research Centre, Near TEC Building
Cherlapally, Hyderabad
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(9) Stanley Browne Laboratory
The Leprosy Mission Community Hospital
Delhi, India

(10) Department of Microbiology, Immunology and Pathology
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The WHO Global Leprosy Programme organized a meeting on "Sentinel Surveillance for Drug Resistance in Leprosy" at Cotonou, Benin on 12-13 November 2012. Representatives from five national programmes, scientists from eight reference laboratories and experts from collaborating centers participated in the meeting. Data on registered relapse cases from five national programmes and information on laboratory findings from eight reference laboratories of the surveillance network were discussed in the meeting.

The conclusions from the meeting emphasized the need for expansion of the surveillance network to improve continuous monitoring of relapse cases in all national programmes, with special focus on treatment completion and drug resistance. It was gratifying to note that rifampicin resistance was rare in all countries reporting data for 2011-2012 and the standard WHO-recommended MDT regimens remain the treatment of choice for leprosy.

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