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Opinion Article

Need and strategy for sentinel surveillance for drug resistance in leprosy in India

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In the fight against leprosy drug resistance poses a serious impediment at a stage when there is dramatic decline in prevalence due to intensive and concerted chemotherapy intervention. Drug resistance in leprosy has been reported since 1964 for dapsone, 1976 for rifampicin and 1996 for ofloxacin. Recent reports and publications have indicated few instances of rifampicin resistance in several endemic areas. In light of reporting drug resistance in leprosy, the National Leprosy Eradication Programme (NLEP) in India has started collecting information on relapse cases from peripheral institutions. The data show quite significant number of relapse cases (328 in year 2008-09) reported from few endemic states. Comprehensive data on the magnitude of drug resistance are crucial to evaluate the efficacy of MDT and to maintain the effectiveness of the current leprosy control strategy. It has become a necessity to develop a surveillance system to keep a close vigil on drug resistance. PCR based assays have convincingly demonstrated that detection of rifampicin resistance by this method is a feasible and practical alternative to the mouse foot pad (MFP) assay and has practical application in India. Surveillance of drug resistance in leprosy can be carried out based on a sentinel surveillance model. Certain district hospitals and tertiary institutions can be identified as sentinel sites in endemic states where tissue samples can be collected and transported to the identified reference laboratories. Based on the suspected and confirmed relapsed cases reported, 12 states have been identified for inclusion under the surveillance of drug resistance in leprosy. These are Andhra Pradesh, Bihar, Chhattisgarh, Karnataka, Madhya Pradesh, Maharashtra, Orissa, Rajasthan, Tamilnadu, Uttar Pradesh, West Bengal and Delhi. Four reference laboratories have already been identified, one each in the states of Uttar Pradesh, Andhra Pradesh, Tamilnadu and Delhi. Tissue samples from sentinel sites would be sent to designated laboratories for conducting the DNA sequencing tests to confirm rifampicin resistance.

Key words : Drug resistance surveillance, Leprosy, India

Introduction

The fight against leprosy has been a great success in India mainly due to introduction of multidrug therapy (MDT) since 1983. Due to availability of free MDT and MDT coverage, the disease prevalence has declined remarkably during current decade. The annual new case detection (ANCD) has also shown decline since the year 2002-03. For leprosy, a chronic disease with social stigma, drug resistance poses a serious impediment at a stage when there is dramatic decline in prevalence due to intensive and

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concerted chemotherapy intervention (WHO 2007). Recent reports have indicated few instances of rifampicin resistance in several endemic areas (Jacobson and Hastings 1976, Guelpa-Lauras et al 1984, Grosset et al 1989, Pfaltzgraff and Ramu 1994, Matsuoka et al 2000, Maeda et al 2001, Matsuoka et al 2003, Norman et al 2003). Resistance to dapsone and clofazimine have also been reported earlier but convincing data supporting existence of clofazimine resistance strains of *M. leprae* have not been reported.

Drug resistance is the reduction in effectiveness of a drug in curing a disease or improving a patient's symptoms. When the drug is not intended to kill or inhibit a pathogen, then the term is equivalent to dosage failure or drug tolerance. More commonly, the term is used in the context of diseases caused by pathogens. Pathogens are said to be drug resistant when drugs meant to inhibit or kill them have reduced effect. When an organism is resistant to more than one drug, it is said to be multidrug resistant (MDR). Secondary or acquired resistance is a result of inadequate chemotherapy characterized by initial clinical improvement followed by regression despite continued treatment. Infection with drug resistant organisms spread by patients with relapsed secondary resistance is defined as primary resistance.

In the light of reports of drug resistance in leprosy, the National Leprosy Eradication Program (NLEP) has started focusing on the problem. The data from different places show quite significant number of relapse cases reported from few endemic states (Table 1). Since rifampicin is the backbone of MDT regimens, it becomes important to monitor the emergence of rifampicin-resistant mutants. It has become a necessity to develop a surveillance system to keep a close vigil on drug resistance.

Mechanism of drug resistance

Microbes do not always make perfect copies of itself with billions of microbes being made every day; lots of small, random differences almost like mistake can happen in any new microbe that gets made. The differences are called mutations. Antibiotic resistance evolves via natural selection acting upon random mutation but it can also be engineered by applying an evolutionary stress on a population. Acquired resistance that develops due to chromosomal mutation and selection is termed vertical evolution. Once such a gene is generated, bacteria can then transfer the genetic information in a horizontal fashion (between individuals).

Drug resistance has been considered as a natural response to the selective pressure of the drug. However, it is exacerbated by several factors, including abuse, under use or misuse of antimicrobials, poor patient compliance and poor quality of available drugs (McManus 1997). There are four major modes of action by antimicrobial agents - (i) interference with cell wall synthesis (ii) inhibition of protein synthesis (iii) interference with nucleic acid synthesis and (iv) inhibition of metabolic pathways. Accordingly, the four main mechanisms by which bacteria exhibit resistance to antibiotics are- (i) drug inactivation (ii) alteration of target sites (iii) alteration of metabolic pathways and (iv) reduced drug accumulation and sporulation. Generally, discontinuation of treatment and monotherapy play a major role in production of MDR bacilli. It is well known that irregular treatment leads to low drug concentrations in blood or tissues. Low drug concentrations provide an opportunity for resistant mutants to survive and further mutate into organisms with an even higher degree of resistance.

Potential causes of drug resistance include -

- Inadequate treatment due to poor drug supply or poor case holding or non adherence of patients to prescribed drug regimen
- Monotherapy
- Poor quality of drug and

- Indiscriminate used of anti-microbial drugs in private sector

Drug resistance in leprosy

Currently two drug regimens have been officially recommended by the WHO -

- MDT-PB schedule containing dapsone and rifampicin
- MDT-MB schedule containing dapsone, clofazimine and rifampicin

Although these regimens are effective, the need for new regimens that are more effective and operationally less demanding is being felt for many reasons. From the operational point of view, the duration of MDT-MB is too long. Two of the components of MDT-MB, dapsone and clofazimine are weak bactericidal and clofazimine has some unpleasant side effects. Besides, as there is no clear indication that leprosy is a disappearing disease; the efforts to control leprosy must be sustained and adapted to the present situation. Resistant *M. leprae* occur spontaneously as a result of mutational events and are present in wild strains of bacterial population that have never been exposed to any drug.

Identification of drug resistance in leprosy

Treated leprosy patients presenting with fresh lesions, yet signs of healing at other sites, suggest possible drug resistance. Much higher bacterial / morphological indices obtained from skin smears of apparently healed areas and fresh lesions support a diagnosis of drug resistance. It is relatively easy to diagnose secondary drug resistance in multibacillary (MB) patients than in paucibacillary (PB) patients.

Drug resistance should be suspected when-

1. There is no response to chemotherapy and development of disease continues.
2. There is relapse of the disease.

Proper identification of relapse cases and to monitor trends over a period of time is essential in the process of controlling drug resistance. A relapse in leprosy is defined as the re-occurrence of the disease at any time after the completion of a full course of treatment. Criteria for relapse in leprosy are as follow -

- Appearance of new skin lesion or new activity in previously existing skin lesions or new nerve function loss or new paralysis of muscle in a patient treated by complete course of chemotherapy.
- The finding of new skin lesion or previous lesion with a high skin smear bacterial index (BI) containing solid staining bacilli. An increase in BI is regarded as the key indication of the multiplication of *M.leprae* and the morphological index (MI) is also considered a useful additional tool in the diagnosis of relapse.
- Histological evidence of relapse in a skin or nerve biopsy where specific changes of leprosy or acid fast bacilli are found.

Relapse is diagnosed by the appearance of definite new skin lesions and/or an increase in the bacillary index (BI) of two or more units at any single site compared to BI taken at the same site at previous examination.

Reported relapse cases in India

NLEP has initiated collection of data on relapse in India since 2007-08. States are submitting data regularly through monthly progress report. The state wise number of relapse cases reported in India in 2008-09 is given in Table 1. However, there is practically very little information of prevalence and incidence of drug resistance in leprosy. Comprehensive data on the magnitude of drug resistance are crucial to evaluate the efficacy of MDT and to maintain the effectiveness of the current leprosy control strategy.

Surveillance of drug resistance

Surveillance of drug resistance in leprosy can be carried out based on a sentinel surveillance model. Certain district hospitals and tertiary institutions can be identified as sentinel sites in endemic states where tissue samples can be

Table 1: Number of relapse cases reported by the states during the year 2008-09

States and UTs	No. of relapse cases
Andhra Pradesh	89
Arunachal Pradesh	0
Assam	9
Bihar	27
Chhattisgarh	62
Goa	11
Gujarat	4
Haryana	5
Himachal Pradesh	7
Jharkhand	2
Jammu & Kashmir	3
Karnataka	64
Kerala	14
Madhya Pradesh	38
Maharashtra	141
Manipur	0
Meghalaya	3
Mizoram	0
Nagaland	0
Orissa	59
Punjab	14
Rajasthan	57
Sikkim	0
Tamilnadu	48
Tripura	2
Uttar Pradesh	112
Uttarakhand	5
West Bengal	109
A & N Islands	2
Chandigarh	3
D & N Haveli	1
Daman & Diu	0
Delhi	0
Lakshadweep	0
Puducherry	5
Total	**896**

Source - MPR, Central Leprosy Division, DGHS, Delhi

collected and transported to the identified reference laboratories.

Rifampicin is the key component in the chemotherapeutic regimens being used to combat leprosy and tuberculosis. The main aim of the sentinel surveillance system should be to detect secondary rifampicin resistance among patients who have relapsed after completing a full course of treatment with MDT for MB leprosy. The inclusion of dapsone resistance would further enhance the preparedness to face rifampicin resistance. At present, molecular methods to identify clofazimine resistance are still not available.

It would be highly desirable to have a rapid, simple technique for monitoring suspected cases of rifampicin resistance that could be applied directly to clinical specimens. During the last 15 years several PCR based assays/ techniques have been developed have been developed for the detection of rifampicin resistance. Application of such methods shows that molecular methods are feasible and practical alternative to the mouse foot pad (MFP) assay and has practical application in countries where leprosy burden is relatively high (Williams and Gillis 2004).

Molecular genetic tests provide a rapid screening tool for detection of majority of resistant isolates. As this test detects only nucleotide mutations, it can not distinguish silent amino acid changes from those that result in amino acid substitution. The rapidity and ease of interpretation of the PCR assay(s) compared with MFP assay is an important finding and supports the potential use of this assay. Published results suggest that MARS assay is rapid and simple to implement and could be performed for detecting rifampicin resistant *M.leprae.* (Guelpa-Lauras et al 1984).

In a study specimens from 59 relapse cases from Colombia (2006-2008), Indonesia (2000-2005) and Myanmar (2005-2007) were tested by DNA sequencing (WHO 2009) and the result shows mutation in 14 cases as given in Table 2.

The sentinel surveillance system

It could comprise of two parts- (i) the first component is the systematic collection of samples from identified relapse cases in the field and transportation of samples to the respective reference laboratory. The specimens can be collected systematically at the identified sentinel

Table 2: Detection of dapsone and rifampicin resistance by DNA sequencing

Country	No. of relapse cases tested	Mutation present against dapsone	Mutation present against rifampicin
Colombia	28	4	2
Indonesia	21	1	2
Myanmar	10	3	2
Total	**59**	**8**	**6**

sites. (ii) the second component is the laboratory part which can be carried out by identified referral laboratories (sentinel surveillance centres) receiving samples from the field and carrying out test for rifampicin and dapsone resistance.

The surveillance activity can be carried out in selected endemic states that are currently detecting significant numbers of new leprosy cases annually. Surveillance activity should be an ongoing activity and participating laboratories will need to take into account the need to maintain the sentinel surveillance work over a period of time. Each sentinel surveillance centre (SSC) can be allotted specified state areas from where tissue specimens could be sent routinely. MB relapse cases referred to the selected referral facilities need to be examined by an expert (designated experienced Dermatologist/ Physician) to confirm the diagnosis of relapse using strict criteria so as to reduce selection bias.

Criteria for inclusion of MB relapse cases

A person who was initially classified as an MB case and has taken at least 12 monthly doses of MB-MDT as recommended by WHO and who is now showing signs and symptoms of relapse without any evidence of lepra reaction. MB classification is based on having 6 and more skin lesions or being skin smear result positive at any single site. From each case, 4 slit-skin smear samples will be required to be collected. Each sample could be taken from a different skin lesion that is most prominent. Tissue scraping collected from a slit-skin smear contains enough bacilli for polymerase chain reaction (PCR) amplification.

Laboratory tests

Nucleotide sequencing of the drug resistance determining region in the *rpoB* for rifampicin and *folP* genes for dapsone respectively can be done using PCR and direct sequencing. Isolates with amino acid substitution in one or more drug resistance determining regions, which have been confirmed to confer rifampicin and dapsone resistance by the mouse footpad method, should be scored as resistant to the respective drug(s).

Quality control of the reference laboratories that are carrying out DNA sequencing for rifampicin resistance could be conducted following standard procedures. A tertiary institute can be identified to conduct quality control in sentinel sites. Quality checks are needed from time to time regarding patient selection, data entry and transportation of specimens.

Reference laboratories (sentinel surveillance centres)

Four reference laboratories have already been identified, one each in the states of Uttar Pradesh (National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Agra), Andhra Pradesh (Blue Peter Research Centre, Hyderabad), Tamilnadu (Schieffelin Institute of Health Research and Leprosy Centre, Karigiri) and Delhi (Stanely Brown Laboratory, Shahdara Leprosy Hospital, The Leprosy Mission, Nand Nagari, Delhi) where tissue samples would be sent from sentinel sites for conducting the DNA sequencing tests to confirm rifampicin and dapsone resistance.

Sentinel sites in selected states

Based on the suspected and confirmed relapsed cases reported, 12 states have been identified for inclusion under the surveillance of drug resistance in leprosy. These are Andhra Pradesh, Bihar, Chhattisgarh, Karnataka, Madhya Pradesh, Maharashtra, Orissa, Rajasthan, Tamilnadu, Uttar Pradesh, West Bengal and Delhi.

Reporting and dissemination of information

Each sentinel surveillance centre (SSC) would be required to take necessary steps to inform the health facility where the MB relapse patient will be under gone treatment for information and further action. Reference labs should also send a copy of the results along with case report forms submitted to them by various participating sentinel sites to tertiary institute identified for quality control, data compilation and analysis every month. Institute would then submit an annual report of all the samples tested and the results to the Global Leprosy Programme, WHO with a copy to the Central Leprosy Division, Government of India, New Delhi.

Management of MB relapse cases

All MB relapse cases included in the surveillance study should immediately be put on treatment with standard MB-MDT without waiting for the results on the status of drug resistance from the SSC Laboratory. If the result comes back as sensitive to rifampicin, MB-MDT treatment is to be continued accordingly. Should patients be reported to be resistant to dapsone only, standard MB-MDT is to be continued. In case the laboratory report that the patient is harbouring rifampicin resistant *M.leprae*, the new regimen of MDT should be given.

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