

## Emergence of primary drug resistance to rifampicin in *Mycobacterium leprae* strains from leprosy patients in India

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Although the prevalence of leprosy has significantly decreased since the introduction of the World Health Organization (WHO) regimen of multidrug therapy (MDT), the incidence remains high, with a report of approximately 215 656 cases globally in 2013. Among these, 126 913 cases were reported from India alone [1]. Of a total number of 3196 relapse cases, India alone contributed 486 cases [1]. For most of the infectious diseases for which secondary prevention is provided by chemotherapy alone, the emergence of drug resistance ultimately becomes a concern and a threat to the intervention programmes. However, the fight against leprosy has been a great success, largely because of the development of MDT in 1981. The efficacy of MDT in curing leprosy during the last three decades has brought about a dramatic decline in the disease burden in all leprosy-endemic countries. The annual new case detection rate has also started to decline in some countries. In India, the prevalence of leprosy decreased from 4.2 per 10 000 population in 2002 to 0.68 per 10 000 population in 2014 [2]. At this stage of elimination (<1/10 000), any emergence of drug-resistant *Mycobacterium leprae* strains will greatly hamper the control programme of the country. Previous records reveal that, after almost 30 years of dapsone monotherapy, dapsone-resistant *M. leprae* was a major issue in the leprosy control programme [3].

As rifampicin is the main drug used in MDT and the only bactericidal drug, it is very important to follow the emergence

of rifampicin-resistant mutants in leprosy patients. It has already been indicated in the recent published literature from Brazil and India [4,5] that rifampicin-resistant cases are appearing in many endemic areas of these countries. To overcome the challenge of containing the disease and to sustain the ongoing declining trend of leprosy in endemic countries, it is essential to monitor drug sensitivity patterns in the present settings. The Leprosy Mission (TLM) Trust India is one of the sentinel centres of the WHO for surveillance of drug resistance in leprosy. The WHO sentinel surveillance was undertaken to identify leprosy patients who already completed an MDT regimen and had relapsed, and did not show any reduction in the activity of their lesions and bacteriological indices (BIs), in spite of re-introduction of MDT. The cases were classified as multibacillary and paucibacillary, according to WHO guidelines. Along with these cases, we enrolled some new cases with high BIs who had not been treated with any MDT before recruitment, to detect primary drug resistance. Primary drug resistance refers to patients who have never been treated for leprosy with MDT.

A total of  $1.27 \times 10^5$  new leprosy cases were detected in India during the period 2013–2014, with an annual new case detection rate of 9.98 per 100 000 population. Of these, approximately 5000 new cases reported to TLM hospitals for treatment.

Written informed consent was obtained from all of the recruited patients, and the study was approved by the Institutional Ethical Committee. A total of 215 slit-skin scrapings from relapsed leprosy patients and new patients were obtained between 2009 and 2014. Slit-skin smears were collected into 70% ethanol in 1.5-mL Eppendorf tubes. The BI of the slit-skin smears varied between 1+ and 6+. DNAs of *M. leprae* were extracted from slit-skin scrapings with the protocol described by Lavania *et al.* [5]. These lysate preparations were further used for DNA sequencing followed by PCR amplification targeting *rpoB*, *folP1*, and *gyrA* [5]. Sterilized distilled water was used as a negative control for PCR, and the reference strain of Thai 53 was used as a positive control. The PCR products were confirmed by 2% agarose gel electrophoresis, and sequence data were analysed with MEGA 5.1.

Among 215 cases, there were 200 multibacillary cases and 15 paucibacillary cases. Among these 215 cases, there were 184 cases of relapse, 16 new cases, and five defaulters. Among the 16 new cases, we observed mutations at already reported codon positions (424, 425, 437, and 438) and at a new codon position (411) in *rpoB* in three patients (Table 1). In the rest of the specimens, we detected secondary resistance mutations in *rpoB* at codons 410 (Glu→Val), 411 (Ala→Val), 424 (Val→Gly), 437 (Ser→Gln), 439 (Phe→Leu), 442 (Gln→His), and 455 (Leu→Pro), which were reported in our previous study [5]. Among these 16 new cases, seven showed primary

**TABLE 1.** Details of primary rifampicin resistance cases

Patient ID	Age (years)/sex	Classification	Clinical presentation	BI	Mutations
PTD 1	60/M	LL	Nodules all over the body, six nerves involved	4+	Ala411Val; Ala425Gly
PTD 2	36/M	LL	Infiltration and nodules all over the body, bilateral claw hand with shortening of the fingers and bilateral sole, complete anaesthesia in the past 6 years	5+	Val424Gly; Gln442His
PTD 3	40/M	BL	Infiltration and nodules all over the body for 1 year	6.0+	Leu455Pro
PTD 4	42/M	LL	Left ulnar weakness, right ulnar paralysis with anaesthesia and right foot weakness and left foot drop for 3–4 months	4.0+	Ala411Val
PTD 5	44/M	LL	Hypopigmented anaesthetic patches present on left thigh, sensory loss present in B/L hand and feet; had never been treated with antileprosy drugs before	1.0+	Ala411Val
PTD 6	48/M	BL	Presented with type 2 reaction	5.33+	Ser437Gln
PTD 7	37/M	BL	Presented with type 1 reaction	4.0+	Gln438Tyr

BI, bacteriological index; BL, borderline lepromatous leprosy; LL, lepromatous leprosy; M, male.

resistance to rifampicin. Of these seven cases, three cases showed a mutation at codon 411 (Ala→Val) that has not been reported previously (Table 1). Patient 1 and patient 2 had mutations at two codon positions. All of these mutations were in the rifampicin resistance-determining region. Genotyping of these isolates showed that they were *M. leprae* type 1D, which we have reported previously in this endemic region [6]. The BIs of all of these new cases varied between 1+ and 6+. Resistance to dapsone was indicated by a mutation at codon 53 (Thr→Ala) in *folP* in one of the new cases (Table 1).

After approximately 30 years of MDT, it is quite natural to expect the emergence of drug resistance in *M. leprae*, and hence, during the stage of elimination, it will be a major setback to the public health programme. Drug-resistant leprosy infection can be caused by the transmission of already resistant strains (primary resistance) or by the selection of resistance-conferring mutations during inadequate therapy (secondary resistance). Lavania *et al.* [5] indicated the presence of rifampicin, dapsone and ofloxacin resistance cases in high-endemicity areas. *M. leprae* isolates that are resistant to single and multiple drugs have been encountered. The emergence of drug resistance to rifampicin will reduce the efficacy of MDT, and will result in a failure to maintain the efficacy of current leprosy control strategies around the world. Although the genome of *M. leprae* has undergone massive gene decay, the rifampicin resistance-determining region of *M. leprae* has been stable, indicating the ability of *M. leprae* to develop resistance under drug pressure. After 30 years of dapsone monotherapy, resistance to dapsone emerged in the 1980s, and it is likely that, after another three decades of rifampicin-based MDT, the emergence of rifampicin resistance might be a major issue in chemotherapy for leprosy. The findings of this study show the emergence of resistance to rifampicin in new cases of leprosy. The emergence of new cases with resistance to rifampicin indicates that resistant strains are actively circulating in endemic regions of India from secondary resistance cases, and infecting the naive population at risk. This finding suggests that there is an urgent need for the establishment of a drug resistance monitoring policy and careful post-treatment follow-up of

cured patients, in order to detect relapse earlier and rapidly identify strains with secondary resistance for their inclusion in a new drug regimen. This report emphasizes the urgent need for the inclusion of new drugs in the multidrug regimen for the treatment of such cases.

## Transparency declaration

The authors state that they have no conflicts of interest.

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