The frequency of drug resistance mutations in *Mycobacterium leprae* isolates in untreated and relapsed leprosy patients from Myanmar, Indonesia and the Philippines

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Summary

Introduction  The magnitude of drug resistance in *Mycobacterium leprae* to dapsone, rifampicin, and ofloxacin was studied in three Southeast Asian countries with a high prevalence of leprosy.

Methods  *M. leprae* from the skin of leprosy patients was collected in North Maluku and North Sulawesi in Indonesia, Yangon in Myanmar, and Cebu in the Philippines. Mutations in the drug resistance determining regions in the *folP1*, *rpoB*, and *gyrA* genes, which have been proven to confer resistance, were analysed. In addition, samples from 51 newly diagnosed cases and 13 patients with leprosy relapse in Cebu were submitted for susceptibility testing in the mouse footpad.

Results  Of 252 isolates obtained from new cases, 3% were dapsone resistant and 2% were rifampicin resistant. In samples taken from patients with relapsed leprosy (n = 53), significantly more resistance mutations were detected: 15% had dapsone resistance mutations, and 8% had rifampicin resistance mutations. Two patients...
with relapsed leprosy had mutations for both dapsone and rifampicin resistance. No mutations conferring quinolone resistance were detected. No mutations were detected in the \textit{folP1} gene of \textit{M. leprae} isolates with a low degree of resistance to dapsone.

\textbf{Discussion} Detection of drug-resistant cases by mutation detection in the drug resistance determining region of the genome is a practical method for monitoring resistance. A comparison of the results obtained in this study with previous data obtained prior to the use of multidrug therapy (MDT), does not indicate clearly whether the magnitude of drug resistance has changed. Larger studies of resistance mutations in \textit{M. leprae} isolated from patients with relapsed leprosy are needed to confirm our results.

\textbf{Conclusion} We recommend monitoring the magnitude of drug resistance globally, by testing \textit{M. leprae} DNA from relapse cases and a representative sample of new cases.

\section*{Introduction}

Multidrug therapy (MDT) was introduced for leprosy control to minimise the development of drug resistance in \textit{Mycobacterium leprae}.\textsuperscript{1} Implementation of MDT in leprosy control markedly decreased the global prevalence of the disease during the last two decades, as expected,\textsuperscript{2} but isolates with resistance to one or more antibiotics have been detected in many areas.\textsuperscript{3–9} Comprehensive data on the magnitude of drug resistance is crucial to evaluate the efficacy of MDT and to maintain the effectiveness of the current leprosy control strategy; the mouse footpad method for drug susceptibility testing is not, however, applicable for large-scale surveillance of the global level of resistance, because it is cumbersome, time-consuming, and available in only a few laboratories in the world. Also, although knowledge of the drug susceptibility of the causative organism of individual patients initiating treatment may be beneficial, the footpad method is impractical for this purpose. Resistance to the anti-leprosy drugs, dapsone, rifampicin and ofloxacin, evolves by amino acid substitution at the binding sites of these drugs. The elucidation of mechanisms for resistance enables us to examine susceptibility to these drugs by a DNA-based assay of PCR-direct DNA sequencing.\textsuperscript{4–16}

In the present study, the frequency of \textit{M. leprae} mutations in the drug resistance determining region (DRDR) in the \textit{folP1}, \textit{rpoB}, and \textit{gyrA} genes, which have been proven to confer resistance to dapsone, rifampicin, and ofloxacin, respectively, were examined. With this methodology, a large number of isolates were tested to obtain useful data for exact analysis of drug resistance levels, and the frequency of drug resistance was determined by pertinent DNA sequencing of \textit{M. leprae} isolates from new and relapse cases in three Southeast Asian countries, namely, Indonesia, Myanmar, and the Philippines.

\section*{Materials and Methods}

\textit{M. leprae} from the skin of leprosy patients was collected in North Maluku and North Sulawesi in Indonesia (2000–2005), Yangon in Myanmar (2003–2005), and Cebu in the Philippines (2001–2006). The samples were obtained from patients before starting MDT (new cases), from patients treated with MDT for up to 4 months (recent cases), and from patients with relapse (defined as patients who developed new skin lesions after the completion of MDT and whose BI had increased by more than 2 log units at any site\textsuperscript{17}).
Bacterial specimens were obtained from the skin lesions of patients by the standard slit skin smear method commonly utilised for assessment of the bacterial index (BI), using a disposable scalpel blade. The material remaining on the blade after doing the smear was used for the study, the blade being soaked in 1 ml of 70% ethanol and kept in a separate vial at room temperature until analysis.

Additionally, *M. leprae* from skin biopsies from 64 patients, including 51 newly diagnosed and 13 relapse cases in Cebu, was submitted for susceptibility testing in the mouse footpad. Groups of mice were infected in both hind footpads with 5000 *M. leprae* and fed continuously a diet containing either no drug, dapsone 0·01%, dapsone 0·001%, dapson 0·0001%, or clofazimine 0·001%, while other mice received rifampicin 10–20 mg/kg/5 times weekly by gastric gavage. Six months after footpad inoculation *M. leprae* was enumerated from those footpads. Drug resistance was deemed to be present when the number of *M. leprae* exceeded 100,000 viable bacilli in drug treated mice. Sequences in the DRDR of each gene were analysed, in DNA recovered from bacilli which grew in the footpads of mice treated with dapsone.

For the analysis of drug resistance by mutation detection, the blades were sent to Japan in separate, labeled tubes and the bacilli-containing tissues were removed from the tip of the blade using a sterile toothpick. One toothpick was used for each blade to avoid cross-contamination. DNA templates were prepared using a previously described method. Mutations in the *folP1*, *rpoB*, and *gyrA* genes were analysed by PCR-direct DNA sequencing. DNA fragments containing codons known to be associated with resistance for dapsone, rifampicin, and ofloxacin were amplified by nested PCR. Nested PCR was carried out using a G mixture of the FailSafe PCR System (EPICENTRE, Madison, WI, USA) in a 25 μl volume.

Primers were designed according to the sequence of *folP1* (accession No. AL583917, *Gene ML0224*), *rpoB* (accession No. AL583923, *Gene ML1891*), and *gyrA* (accession No. AL583917, *Gene ML0006*) of *M. leprae*. The sequences of the primers are listed in Table 1.

DNA fragments corresponding to the whole *folP1* gene of isolates found to be dapsone resistant to a low degree, were sequenced as described by Kai *et al.* The PCR programme consisted of one hold cycle of 2 min at 94 °C linked to a three-step cycle of 30 s at 94 °C, and 30 s at 56 °C, and 30 s at 72 °C for 30 cycles followed by a final hold cycle of 5 min at 72 °C. PCR fragments were purified and sequenced according to the same protocol as previously described.

**Table 1.** Sequences of oligonucleotide primers for *M. leprae*

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>folP1</em> gene</td>
<td></td>
</tr>
<tr>
<td>Outer primers</td>
<td>folP1-F1</td>
</tr>
<tr>
<td></td>
<td>folP1-R1</td>
</tr>
<tr>
<td>Inner primers</td>
<td>folP1-F2</td>
</tr>
<tr>
<td></td>
<td>folP1-R2</td>
</tr>
<tr>
<td><em>rpoB</em> gene</td>
<td></td>
</tr>
<tr>
<td>Outer primers</td>
<td>rpoB-F1</td>
</tr>
<tr>
<td></td>
<td>rpoB-R1</td>
</tr>
<tr>
<td>Inner primers</td>
<td>rpoB-F2</td>
</tr>
<tr>
<td></td>
<td>rpoB-R2</td>
</tr>
<tr>
<td><em>gyrA</em> gene</td>
<td></td>
</tr>
<tr>
<td>Outer primers</td>
<td>gyrA-F1</td>
</tr>
<tr>
<td></td>
<td>gyrA-R1</td>
</tr>
<tr>
<td>Inner primers</td>
<td>gyrA-F2</td>
</tr>
<tr>
<td></td>
<td>gyrA-R2</td>
</tr>
</tbody>
</table>
Isolates with mutations at codons 53 and 55 in the \textit{folP1} gene, at codons 407, 410, 420, 425, 427, in the \textit{rpoB} gene, and at codon 91 in the \textit{gyrA} gene were defined as resistant to dapsone, rifampicin, and ofloxacin, respectively. These mutations have been confirmed to confer resistance to the drug, dapsone,\(^5,6,8,15,16\) rifampicin,\(^4,6,8,10,13\) or quinolone\(^4,6,8\) by the mouse footpad susceptibility test and by mutation detection in the DRDR for each drug. Frequencies of resistance were compared by the Fisher’s exact test.

The study was approved by the institutional ethics committee of the National Institute of Infectious Diseases, Japan, and the three local institutional review boards. Informed consent was obtained prior to the collection of bacterial samples.

Results

Biopsies and slit skin smears were analysed from 121 new or recent cases and 10 relapse cases from Indonesia, 54 new or recent cases and 24 relapse cases from Myanmar, and 77 new or recent cases and 19 relapse cases from Cebu. All newly detected cases were treated with WHO MDT.\(^{21}\) Almost all patients in Indonesia who relapsed were retreated with same WHO regimen. The MB patients who relapsed in Cebu, were retreated with monthly doses of rifampicin 600 mg, ofloxacin 400 mg and minocycline 100 mg for a total of 12 doses. In Myanmar, when susceptibility test results were known after relapse, patients with susceptible bacilli were treated with same WHO regimen. If dapsone resistance was found, patients were treated with clofazimine 300 mg monthly, clofazimine 50 mg daily, and rifampicin 600 mg monthly for one year. In general, these patients have responded well to the alternative treatment, although final follow-up details are not yet available.

The frequency of drug resistance to the three antibiotics studied varied between countries, and between new and relapse cases (Table 2).

In Indonesia, dapsone resistance mutations was found in 1/121 (1%) new and recent cases and 1/10 (10%) relapse cases; in Myanmar, in 4/54 (7%) new and recent cases and 2/24 (8%) relapse cases; and in the Philippines in 2/77 (3%) new cases and 5/19 (26%) relapse cases. In Indonesia, 4/121 (3%) of \textit{M. leprae} isolates from new and recent cases were found to have rifampicin resistant mutations, while 2/10 (20%) relapse cases were found to have rifampicin resistant mutations. In Myanmar, 1/54 (2%) \textit{M. leprae} isolates from new and recent cases were found to have rifampicin resistant mutations, while isolates from 2/24 (8%) relapse cases had rifampicin resistance mutations. In the Philippines, 0/77 (0%) \textit{M. leprae} from new and recent cases had rifampicin resistance mutations and 0/19 (0%) relapse cases had

<table>
<thead>
<tr>
<th>Country</th>
<th>New or recent case</th>
<th>Relapse case</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dapsone</td>
<td>Rifampicin</td>
</tr>
<tr>
<td>Indonesia (North Maluku and North Sulawesi)</td>
<td>1/121 (0.8%)</td>
<td>4/121 (3.3%)</td>
</tr>
<tr>
<td>Myanmar (Yangon)</td>
<td>1/54 (1.8%)</td>
<td>0/54</td>
</tr>
<tr>
<td>Philippines (Cebu)</td>
<td>2/77 (2.6%)</td>
<td>0/77</td>
</tr>
</tbody>
</table>
rifampicin resistance mutations. The frequency of resistance mutations for both dapsone and rifampicin was consistently higher in patients with leprosy relapse than in new cases, in each of the areas studied. In fact, the frequency of both dapsone and rifampicin resistance mutations was significantly higher in the full cohort of relapse cases than in new and recent cases, \( P < 0.001 \) and \( P < 0.05 \) respectively. Ofloxacin resistance was not evaluated in patients in Indonesia, and was found in no new cases (131) or patients with relapse (43) in Myanmar or the Philippines.

Dapsone resistance mutations in isolates in new or recent cases were detected in all three areas. Four isolates with rifampicin resistance mutations were detected in Indonesia and one in Myanmar, among new or recent cases. An isolate with dapsone resistance mutations was found in an Indonesian patient treated for 2 months. Of four patients with dapsone resistance mutations in Myanmar, three were collected before the start of MDT, and one was from a patient treated for 2 months. Two isolates with dapsone resistance mutations were obtained from patients in Cebu before starting MDT. Of four new or recent cases in Indonesia with rifampicin resistance mutations, one sample was obtained before the start of MDT, two were from patients treated for 2 months, and one was from a patient treated for 4 months with MDT. One isolate from a newly diagnosed case in Myanmar had rifampicin resistance mutations. One isolate in Myanmar and another in Indonesia, both from patients with relapse, had both dapsone and rifampicin resistance mutations. No Multidrug resistance was detected other than these two cases, in all three areas.

The mutations detected were as follows. Mutations in the \( \text{folP1} \) gene included one case of ACC to GTC (Thr \( \rightarrow \) Val) and one case ACC to AGA (Thr \( \rightarrow \) Arg) at codon 53; seven cases of CCC to CTC (Pro \( \rightarrow \) Leu), two cases to TCC (Pro \( \rightarrow \) Ser), two cases to CGC (Pro \( \rightarrow \) Arg), and two cases to CGT (Pro \( \rightarrow \) Arg) at codon 55. Mutations in the \( \text{rpoB} \) gene included one case of GAT to TAT (Asp \( \rightarrow \) Tyr) at codon 410, one case of CAC to GAC (His \( \rightarrow \) Asp) at codon 420; six cases of TCG to TTG (Ser \( \rightarrow \) Leu) and one case of TCG to ATG (Ser \( \rightarrow \) Met) at codon 425. The high frequency of the mutation TCG to TTG at codon 425 is the same result as previously observed in other areas.\(^{10,12}\) No mutation was demonstrated in the \( \text{gyrA} \) gene of isolates from any area.

Of 64 isolates tested by the mouse footpad method, one isolate had dapsone resistance mutations to a high degree (HD), two had dapsone resistance mutations to an intermediate degree (ID), and 5 had dapsone resistance mutations to a low degree (LD) (Table 3).

**Table 3.** The results of susceptibility testing for dapsone by the mouse footpad method and sequencing of the \( \text{folP1} \) gene in *M. leprae*

<table>
<thead>
<tr>
<th>Isolate</th>
<th>DHPS substitution</th>
<th>( \text{folP1} ) mutation</th>
<th>Degree of resistance</th>
<th>Mouse footpad method results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0001% 0.001% 0.01% Controls</td>
</tr>
<tr>
<td>01Mat02</td>
<td>Thr53Val</td>
<td>ACC53GTC</td>
<td>High</td>
<td>5/5 5/5 5/5 5/5</td>
</tr>
<tr>
<td>NCR</td>
<td>Thr53Arg</td>
<td>ACC53AGA</td>
<td>Intermediate</td>
<td>5/5 6/6 0/6 7/7</td>
</tr>
<tr>
<td>02Mat47</td>
<td>Pro55Leu</td>
<td>CCC55CTC</td>
<td>Intermediate</td>
<td>5/6 4/4 0/5 6/6</td>
</tr>
<tr>
<td>01Mat01</td>
<td>None</td>
<td>None</td>
<td>Low</td>
<td>5/5 0/3 0/6 3/6</td>
</tr>
<tr>
<td>01Mat03</td>
<td>None</td>
<td>None</td>
<td>Low</td>
<td>5/8 0/7 0/7 8/8</td>
</tr>
<tr>
<td>02Mat25</td>
<td>None</td>
<td>None</td>
<td>Low</td>
<td>4/5 0/5 0/5 5/5</td>
</tr>
<tr>
<td>EER</td>
<td>None</td>
<td>None</td>
<td>Low</td>
<td>5/7 0/5 0/8 11/11</td>
</tr>
<tr>
<td>MMR</td>
<td>None</td>
<td>None</td>
<td>Low</td>
<td>3/5 0/6 0/6 6/6</td>
</tr>
</tbody>
</table>
The mutation ACC to GTC at codon 53 in the \textit{folP1} gene was detected in the HD isolate, while mutations ACC to AGA at codon 53, and CCC to CTC at codon 55 were detected in the ID isolates. No mutation was demonstrated at either codon in the \textit{folP1} gene of the five isolates with LD in Cebu.

\textbf{Discussion}

The proportion of isolates with dapsone resistance mutations among new and recent cases was 0.8%, 7.2% and 2.6% in Indonesia, Myanmar and the Philippines, respectively. These frequencies of primary dapsone resistance, though of some concern in Myanmar, are generally low, as previously found in San Francisco\cite{22} and the Philippines\cite{23}, and are far lower than the almost 1/3 of cases found in Louisiana\cite{24}, Ethiopia\cite{25} and later in the Philippines\cite{26}, the latter groups being almost entirely LD resistance without known mutation of the \textit{folP1} gene. While the number of patients with leprosy relapse assessed for dapsone resistance in this study was small, fully 8/53 (15%) were found to harbour dapsone-resistant genes. Though this frequency is high, except for the two relapse cases with both dapsone and rifampicin resistance, the reinstitution of WHO MDT, containing the only bactericidal agent in that regimen, rifampicin, currently recommended by the WHO for leprosy relapse following MDT,\cite{21} would likely prove effective. It is unclear whether or not the frequency of dapsone resistance has declined since the wide implementation of MDT, since prior to that time the majority of patients with isolates with dapsone resistance mutations harboured LD strains in many areas\cite{24-26} for which there is no identifiable mutation in the \textit{folP1} gene.

Dapsone resistance in \textit{M. leprae} is known to be the result of specific mutations in codons 53 and 55 within the \textit{folP1} gene coding dihydropteroate synthase (DHPS).\cite{5,6,8,9,15,16} Cambau \textit{et al.} showed that of 10 HD or ID isolates with dapsone resistance mutations, 9 isolates harboured mutations at codon 53 or 55, while one ID isolate showed no mutation in the \textit{folP1} gene.\cite{9} Of 6 LD dapsone resistant isolates, five isolates showed no mutation and one showed a mutation at codon 53.\cite{9} No mutation was detected in 22 susceptible isolates.\cite{9} In other studies, all 15 HD isolates with dapsone resistance mutations showed mutations at codon 53 or 55, while 7 susceptible strains showed no mutation in the \textit{folP1} gene.\cite{5,6,8,15,16} Five LD isolates from the Philippines in our study harboured no mutation in the \textit{folP1} gene. These were all resistant in the mouse foot pad to 0.0001% dapsone in the diet, but not to higher levels. Therefore, almost all isolates identified as dapsone resistance by mutation detection are resistant to dapsone to a high or intermediate degree. However almost no low degree isolates with dapsone resistance mutations could be detected as resistant by mutation detection. Though Shepard\cite{27} found that \textit{M. leprae} obtained from untreated leprosy patients in an earlier era were consistently inhibited by 0.0001% dapsone in diet, Rees\cite{28} found a few were not inhibited at that level. The finding that isolates in the mouse with resistance to dapsone at a concentration of 0.0001% is not associated with a mutation in the \textit{folP1} gene suggests perhaps that resistance to that level of dapsone is found at the far extreme of the dapsone-sensitive \textit{M. leprae} distribution. This concept is important as the vast majority of previously identified dapsone resistance, both primary and secondary, was found resistant only to this level and not higher ones. In any event, it is considered that such cases have no clinical significance, since administration of 0.0001 g DDS per 100 g mouse diet is of the same order as that observed in humans receiving 1 mg DDS daily\cite{29} and the usual dosage of DDS in MDT is 100 mg daily.
As all isolates with HD or ID isolates with dapsone resistance mutations exhibited mutations at codon 53 or 55, the PCR direct sequencing method will detect all clinically significant dapsone resistant cases and the method is feasible for detecting isolates with dapsone resistance mutations.

Although two patients with dapsone resistance mutations among new or recent cases were treated for 2 months with MDT, they can be classified as primary dapsone resistant cases, since the multiplication of the bacilli is very slow. Resistant strains could not replace susceptible strains in the patient within such a short time.

A striking finding of the study is the detection of isolates with rifampicin resistance mutations amongst patients newly or recently detected and a greater frequency amongst relapse cases. Though in the areas studied the rate of primary rifampicin resistance, 2%, is reasonably low, the rate in patients with leprosy relapse, 8%, is of concern, as well as the two relapse cases who were resistant to both dapsone and rifampicin. In these two instances, retreatment with WHO MDT would need to be prolonged for the improvement of condition since MDT for these cases is monotherapy with clofazimine. Though the number of cases with leprosy relapse in this study is small and as previously mentioned, rifampicin is the key and sole bactericidal component of MDT, perhaps reconsideration of an alternative treatment for those who relapse after MDT is in order; this might reasonably include minocycline and moxifloxacin. Larger studies of rifampicin resistance mutations in relapse cases are needed to ascertain if our current results are generally representative.

Rifampicin resistance is conferred by mutations in the beta subunit of RNA polymerase coded by the \( rpoB \) gene. Mutations at codon 407, 410, 420, 425 and insertions between 408 and 409 have been confirmed as associated with rifampicin resistance. Mutations at codons 401, 7, 416, and 427 have also been found but it has not been revealed clearly whether these mutations confer rifampicin resistance in \( M. leprae \). Although mutations at 401 were detected in the \( rpoB \) gene of rifampicin resistance isolates, it is not proven whether this mutation is associated with rifampicin resistance or not, since the mutation occurred simultaneously with a mutation at codon 420 which is known to be associated with rifampicin resistance. Mutations at 416 were also detected but no confirmatory data from the mouse foot pad susceptibility tests were available, although it is known that this mutation confers rifampicin resistance in \( M. tuberculosis \). A mutation at codon 427 was detected in one clinical isolate and one rifampicin resistant isolate. The former case was not confirmed by the mouse footpad method and the latter one was detected concordantly with a mutation at 425, although the mutation at this position is known to be associated with rifampicin resistance in \( M. tuberculosis \). Clarification of the association between these mutations and rifampicin resistance by the mouse footpad method is highly recommended. In studies so far reported on the association of rifampicin resistance with mutations in the \( rpoB \) gene, only one rifampicin resistant isolate showed no mutation. Taking these results into consideration, detecting rifampicin resistant cases by mutation detection in the \( rpoB \) gene is a practical method for monitoring resistance to rifampicin.

The prevalence of rifampicin resistance mutations in cases with leprosy relapse was higher than the incidence in new cases, so this also must be monitored carefully. A previous study showed that among a total of 404 multibacillary patients who had been treated with various rifampicin containing regimens, 22 (5.4%) were resistant to rifampicin. Although a small sample size, the prevalence of rifampicin resistance mutations in Indonesia and Myanmar is higher than that found previously, before MDT was implemented. Two possible
reasons for a high prevalence of drug resistance are poor compliance, both with self-
administration of drugs and premature discontinuation of therapy, and prior monotherapy
with rifampicin, either for leprosy or as part of the standard chemotherapy for tuberculosis
which would also expose leprosy patients to rifampicin monotherapy.

Of the two patients with leprosy relapse with doubly resistant mutations (dapsone and
rifampicin), the one from Myanmar had previously received monotherapy with dapsone
with isolates with dapsone resistance mutations in the Philippines, three had received prior
dapsone, one as monotherapy, and two others either as the sole agent or in one instance
combined with clofazimine and in another combined with clofazimine and rifampicin.
Though the other relapse patients in the Philippines, as well as those from Indonesia and
Myanmar, were treated with WHO MDT, it is unclear whether patients had adhered to the
regimen and completed therapy.

Five isolates with ofloxacin resistance mutations have been reported, all isolates
having the mutation GCA to GTA (Ala → Val) at codon 91 (numbering system as used for
M. leprae) in gyrA gene. A strain with the mutation GGC to TGC (Gly → Cys) at codon 89
was reported previously, but resistance to ofloxacin was not confirmed by the mouse footpad
method. Two other amino acid changes, Ser at 91, and Asp at 94 (numbering system as used
for M. tuberculosis), in the gyrA gene of M. tuberculosis are associated with quinolone
resistance. It seems mutations at the codons 89, 92, and 95 in the gyrA gene of M. leprae
also cause quinolone resistance. No mutation at these codons, 89, 91, 92, and 95, was detected
in the samples tested. Thus the level of quinolone resistance in the areas investigated is still
very low.

The study indicated the existence of primary and secondary resistance to dapsone and
rifampicin in countries where many leprosy cases are still detected. A comparison of the
results obtained in this study with previous data obtained prior to the use of MDT, does
not indicate clearly whether the magnitude of drug resistance has changed. We
consider this study as a first effort to assess the magnitude of drug resistance in the MDT
era. In order to preserve the efficacy of MDT and prevent the spread of drug resistant
bacilli, carefully designed global studies are recommended, as suggested previously.
Monitoring the susceptibility of isolates from each case of leprosy relapse allows optimal
treatment to be chosen, by avoiding ineffective drugs and choosing effective compounds.
The longitudinal surveillance of levels of drug resistance in new cases in some areas with
a high prevalence of leprosy might contribute to predicting the spread of drug resistant
strains. The application of the susceptibility test by mutation detection should be
attempted, especially in cases where treatment failure seems a possibility.

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