Drug Resistance in Patients With Leprosy in the United States

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Molecular drug susceptibility testing was performed on 39 US patients with leprosy. Of these, 2 had dapsone-resistant Mycobacterium leprae and 1 of these patients also had rifampin-resistant M. leprae. Even though antileprosy drug resistance occurs in this leprosy population, resistance does not appear to be a major problem.

Keywords. leprosy; drug resistance; United States.

Although resistance of Mycobacterium leprae to antileprosy drug therapy has been observed in many leprosy-endemic regions of the world (eg, India, Brazil, Southeast Asia, Western Pacific, and Africa) [1, 2], global prevalence rates have not been well documented. Recently, a Global Sentinel Surveillance for Drug Resistance in Leprosy program was established and coordinated by the World Health Organization to monitor global leprosy drug resistance in relapse cases [3]. In 2010, this program reported 9 cases of dapsone-resistant leprosy and 1 case of rifampin-resistant leprosy among 72 patients with relapsed leprosy from 8 participating countries: Mali (African Region), Colombia (American Region), Pakistan and Yemen (Eastern Mediterranean Region), India and Myanmar (South-East Asia Region), and China, Philippines, and Viet Nam (Western Pacific Region) [3]. However, this program does not include countries of low incidence, such as the United States (approximately 200 cases/year), even though the majority of US patients have origins in many of the areas of high endemicity where drug resistance has been identified [4].

The primary agents used for multidrug therapy for leprosy are dapsone, rifampin, and clofazimine; second-line agents include minocycline, clarithromycin, and ofloxacin/levofloxacin. Resistance to dapsone and rifampin has been observed in US patients [5, 6]. The initial findings that drug-resistant leprosy occurs in the United States, a country where the disease is rare, is a cause for concern [4]. However, the rates of resistance in this patient population are unknown. Determining leprosy drug resistance trends in the United States is important because early case detection and multidrug therapy are currently the only effective means to control this disease. Therefore, this study was conducted to begin to determine the rates of antileprosy drug resistance in US patients with leprosy.

Preliminary work at the National Hansen’s Disease Programs (NHDP) Laboratory Research Branch and other laboratories around the world have identified the molecular mechanisms of the resistance of M. leprae to dapsone, rifampin, and ofloxacin [2]. This has led to the development of universal polymerase chain reaction (PCR)/direct DNA sequencing assays, which can detect the drug susceptibility of M. leprae directly from clinical specimens [7]. These assays were used in the current study to obtain preliminary data concerning the rates of drug resistance among US patients with leprosy who were referred to the NHDP for leprosy diagnosis in 2011–2012. The NHDP routinely receives biopsy specimens for histopathologic diagnosis, and specimens have been referred for molecular studies for various reasons, such as when M. leprae cannot be identified histologically because infection of nerves could not be determined, if the patient had a history of incomplete treatment elsewhere, or if there was reason to suspect relapse of infection.

DNA was extracted from paraffin-embedded sections or ethanol-fixed biopsy specimens, using DNeasy Blood and Tissue Kit (QIAGEN). Resultant DNAs were initially tested for the presence of M. leprae-specific RLEP sequences, using a quantitative RLEP real-time PCR (qPCR) assay [8]. Specimens containing M. leprae DNA in the RLEP qPCR assay from 50 patients were further tested for the presence of mutations in the drug resistance–determining regions (DRDRs) of rpoB (associated with rifampin resistance) and folP1 (associated with dapsone resistance), using M. leprae DRDR primers and PCR/direct DNA sequencing assays [7]. Alignment of DNA DRDR sequences from samples with that of the drug-susceptible TN strain of M. leprae [9] was accomplished using ClustalW DDBJ software [10], and mutations were identified. When resistance to either of these drugs was observed, the DRDR of the M.
leprae gyrA gene was amplified and sequenced for identification of mutations associated with resistance to ofloxacin, a second-line drug for leprosy [2].

Both rpoB and folP1 DRDRs were successfully amplified from 39 patients. These included 3 of 10 patients (30%) with paucibacillary leprosy and 36 of 40 (90%) with multibacillary leprosy. These patients had origins in the Western Pacific Islands (18), Central or South America (5), Asia (2), and the United States (14). DNA sequencing of DRDRs demonstrated that 2 of these patients harbored M. leprae containing folP1 mutant alleles, of whom 1 also contained a rpoB mutant allele (Table 1) [6]. All mutations observed in these specimens have previously been associated with dapsone- and rifampin-resistant leprosy [2]. No mutations were observed in the gyrA DRDR of specimens tested, indicating that these patients harbored ofloxacin-susceptible M. leprae.

Both patients with drug-resistant leprosy had origins in American Samoa (Western Pacific Islands). However, rates of antileprosy drug resistance in this region are currently unknown.

In summary, this study showed that low levels of antileprosy drug resistance were detected in the US patients tested. While caution must be taken with the interpretation of these data because of the low sample number and the fact that this was not a prospective study with a standardized sampling method, these data suggest that resistance to antileprosy drugs is not a major problem for leprosy control in the United States. Notably, the occurrence of new lesions in patients with leprosy during or after treatment is usually due to leprosy reactions, which are common, rather than due to relapse, which is rare [11]. A recent retrospective review of leprosy treatment in the United States found no relapses after 10–15 years of follow-up in patients who were compliant with the NHDP treatment protocol [11]. However, 1 of the 2 cases reported here presented as leprosy relapse due to primary drug-resistant [6]; the other case had no history of antileprosy drug therapy before the initial biopsy. Globally, however, drug resistance is often associated with relapse of leprosy. However, because of its very long incubation time, great delays in diagnosis due to nonrecognition by physicians, and the poor history of compliance among some groups migrating to the United States [12], M. leprae drug resistance will be easily overlooked unless monitoring is done. Even though the sensitivity of the molecular assays for detection of drug resistance in paucibacillary lesions containing rare bacilli was lower than that for multibacillary lesions, we recommend biopsy and evaluation for all suspected relapse cases, especially those that have origins where drug resistance is known to occur. The NHDP can assist in differentiating reactions from relapse and thereby potentially improve patient care and contribute to the knowledge of drug resistance levels not only in the US patient population but globally, as well.

Note

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References


Table 1. Results of Molecular Drug Susceptibility Testing of 39 US Patients With Leprosy

<table>
<thead>
<tr>
<th>Variable</th>
<th>Susceptibility, a No.</th>
<th>Resistance, b No. (Mutant Allele(s))</th>
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<tbody>
<tr>
<td>DRDR of folP1 for determining dapsone resistance</td>
<td>37</td>
<td>2 (Thr53Ala or Thr53Ile)</td>
</tr>
<tr>
<td>DRDR of rpoB for determining rifampin resistance</td>
<td>38</td>
<td>1 (Ser425Leu)</td>
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Abbreviations: DRDR, drug resistance-determining region; M. leprae, Mycobacterium leprae.

a Defined as a DNA DRDR sequence associated with the drug-susceptible phenotype of M. leprae.
b Defined as a DNA DRDR containing a mutation associated with the drug-resistant phenotype of M. leprae.
c Defined as specific amino acid changes resulting in a dapsone-resistant phenotype (alanine or isoleucine substituted for threonine in codon 53 of the folP1 DRDR) or a rifampin-resistant phenotype (leucine substituted for a serine in codon 425 of the rpoB DRDR) [2]. One patient containing the dapsone-resistant phenotype (Thr53Ala) also contained Ser425 mutant alleles [6].