REVIEW

Molecular detection of drug resistance in *Mycobacterium leprae*

DIANA L. WILLIAMS & THOMAS P. GILLIS

*Molecular Biology Research Department, Laboratory Research Branch, Division of the National Hansen’s Disease Programs, Baton Rouge, LA, USA*

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Introduction

Treating infectious diseases with combinations of effective antibiotics is a proven method of limiting the emergence and spread of new or existing antibiotic resistant pathogens. Multi-drug therapies (MDT) formulated for leprosy have been based on this principle, and appear to be effective at both minimizing the development of drug resistance as well as reducing the prevalence of leprosy in many areas of the world.1–3 In contrast, MDT has not had an impact on reducing the incidence of leprosy in many parts of the world.4 Establishing the success of a strategy like MDT for leprosy control requires thorough evaluation of treatment failures, including drug susceptibility testing. The current ‘gold standard’ for testing drug susceptibility of *Mycobacterium leprae* relies on the cumbersome and time-consuming mouse footpad assay, which is available only in a few laboratories around the world and can require up to a year to obtain results. Accordingly, drug susceptibility testing is not a routine component of most leprosy control programs, limiting our knowledge of current levels of drug resistance globally.

Over the past several years rapid, DNA-based assays for detecting drug resistant *M. leprae* have been developed.5–9 Even though the assays are based on modern molecular biology techniques, many reference laboratories in leprosy endemic countries have the capability of implementing and utilizing them. Information gained from their implementation could be used as an integral component of an overall public health strategy for monitoring and limiting the spread of drug resistant *M. leprae*. The purpose of this review is to describe the drugs currently used to treat leprosy, the mechanisms of resistance to these drugs found in *M. leprae*, and the DNA-based assays that have been developed to detect drug resistance.

Correspondence to: D. L. Williams, Molecular Biology Research Department, Laboratory Research Branch, Division of the National Hansen’s Disease Programs at Louisiana State University, School of Veterinary Medicine, Room 3517W, Skip Bertman Drive, Baton Rouge, LA 70803, USA (Tel: +1-225-5789839, Fax: +1-225-5789856; e-mail: dwill21@lsu.edu)
Anti-leprosy drugs and resistance mechanisms

In the 1950s, dapsone was introduced as standard chemotherapy for leprosy and was used worldwide for treatment of both multibacillary and paucibacillary forms of the disease. The use of dapsone required long-term, often life-long, treatment to control infections because of its slow bacteriostatic effect on M. leprae. Long-term monotherapy with dapsone resulted in poor compliance in many areas, ultimately leading to treatment failures and the emergence of dapsone resistant strains of M. leprae in the 1970s. This presented serious problems for leprosy control programmes, as resistance levels were reported as high as 40% in some areas of the world. By the mid-1970s it was clear that life-long dapsone monotherapy was failing. Between the 1960s and 1970s, additional antimicrobial agents, such as rifampin and clofazimine, were introduced for the treatment of leprosy. Although rifampin proved to be a powerful anti-leprosy drug, use of rifampin alone or in combination with dapsone for the treatment of dapsone-resistant leprosy led to the rapid development of rifampin-resistant organisms. In addition, clofazimine proved to be only weakly bactericidal against M. leprae and, therefore, was not a suitable single drug therapy for leprosy.

To overcome the threat posed by the worldwide spread of dapsone resistance and to improve treatment efficacy, the World Health Organization recommended multidrug therapy for leprosy in 1981. Regimens included: daily dapsone (100 mg) and clofazimine (50 mg), with monthly rifampin (600 mg) and clofazimine (300 mg) for a duration of 2 years in the treatment of multibacillary leprosy (skin smears with a bacterial index of 2+); and daily dapsone (100 mg) and monthly rifampin (600 mg) used for a duration of 6 months to treat patients with paucibacillary leprosy (skin smears with a bacterial index of 2+). These drug formulations were incorporated into blister packs that could be stored at room temperature. This made it possible to distribute drugs sufficient for several months of treatment to patients in rural or hard to reach locations improving treatment completion rates. With the idea of further enhancing treatment compliance, some have recommended that the treatment for multibacillary leprosy be shortened to 12 months.

MDT for leprosy has been very practical and successful for both multibacillary and paucibacillary leprosy, and the overall prevalence rates of leprosy in the world have fallen dramatically. More recently, ofloxacin and minocycline have been added to the drug arsenal for treating leprosy. It has been recommended that a single dose of a combination ROM which consists of rifampin (600 mg), ofloxacin (400 mg) and minocycline (100 mg) is an acceptable and cost-effective alternative regimen for the treatment of single lesion paucibacillary leprosy. However, even with these powerful drug combinations, drug resistance is still found. A recent report demonstrated that 19% of 265 M. leprae isolates from biopsies of leprosy patients were resistant to various concentrations of dapsone, rifampin or clofazimine and 6-23% were resistant to more than one drug using the mouse foot pad susceptibility assay. In addition, several investigators have identified multidrug resistant strains of M. leprae.

Dapsone

Dapsone (4,4-diaminodiphenyl sulphone) is a synthetic sulphone, is structurally and functionally related to the sulphonamide drugs, and targets dihydropteroate synthase (DHPS), a key enzyme in the folate biosynthesis pathway in bacteria and some eukaryotes. Dapsone inhibits folic acid biosynthesis by acting as a competitive inhibitor
of p-aminobenzoic acid (PABA). Superimposed on this background, specific mutations within the highly conserved PABA binding site of E. coli’s DHPS, encoded by folP, result in the development of dapsone resistance. New evidence from the M. leprae genome sequencing project indicated that M. leprae possesses two folP homologues (folP1 and folP2). folP1 appears to be part of an operon containing three other genes involved in folate biosynthesis which are similar to that of M. tuberculosis. Through surrogate genetic studies with M. smegmatis, the relationship between dapsone resistance and the DHPS of M. leprae has been established. Missense mutations within codons 53 and 55 of the sulphone resistance determining region (SRDR) of folP1 result in the development of high-level dapsone resistance in M. leprae (Table 1).

RIFAMPIN

Rifampin 3-[(4-methyl-1-piperazinyl)-imino]-methyl] rifamycin is the key bactericidal component of all recommended antileprosy chemotherapeutic regimens. A single dose of 1200 mg can reduce the number of viable bacilli in a patient’s skin to undetectable levels within a few days of treatment. The target for rifampin in mycobacteria and E. coli is the beta (β) subunit of the RNA polymerase encoded by rpoB. Comparison of the deduced primary structures of β-subunit proteins from several bacteria to that of M. leprae

Table 1. Mutations within drug target genes associated with drug resistance in Mycobacterium leprae

<table>
<thead>
<tr>
<th>Drug/target gene</th>
<th>Drug susceptibility^a</th>
<th>Mutation</th>
<th>No. isolates (%)^b</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Rifampin/rpoB</td>
<td>R</td>
<td>Gly401Ser; His420Asp</td>
<td>1 (2)</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>Glu407Val</td>
<td>1 (2)</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>Phe408/Met409; LysPhe insert</td>
<td>1 (2)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>NC</td>
<td>Asp410Asn</td>
<td>1 (2)</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>NC</td>
<td>Asp410Asn; Leu427Pro</td>
<td>1 (2)</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>Ser416Cys</td>
<td>1 (2)</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>His420Asp</td>
<td>1 (2)</td>
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<td>R</td>
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<td>11 (20)</td>
<td>27</td>
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<tr>
<td></td>
<td>R</td>
<td>Ser425Leu</td>
<td>33 (60)</td>
<td>5, 7, 9, 27–29</td>
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<tr>
<td></td>
<td>R</td>
<td>Ser425Met</td>
<td>1 (2)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>Ser425Met; Leu427Val</td>
<td>1 (2)</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>Ser425Phe</td>
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<td></td>
<td>NC</td>
<td>Ser425Trp</td>
<td>1 (2)</td>
<td>27</td>
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<td>Dapson/folP1</td>
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<td>12 (40)</td>
<td>27, 37, 38</td>
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<td></td>
<td>NC</td>
<td>Thr53Ala; Pro55Leu</td>
<td>1 (3)</td>
<td>27</td>
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<td></td>
<td>R</td>
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<td>Thr53Ile</td>
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<td></td>
<td>R</td>
<td>Pro55Arg</td>
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<td>Pro55Leu</td>
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<td>27, 37, 38</td>
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<td></td>
<td>R</td>
<td>Ala91Val</td>
<td>6 (86)</td>
<td>27, 28</td>
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</table>

^a R = resistant phenotype as determined by mouse footpad or radiorespirometry (Buddemeyer) drug susceptibility analysis. NC = not confirmed by either assay.

^b Percent (rounded to the nearest whole number) isolates in each drug-resistant group that contain a specific mutation.
demonstrated that *M. leprae* shares six highly conserved functional regions common to this enzyme in bacteria.\(^5,40\)

Mycobacterial resistance to rifampin correlates with changes in the structure of the β-subunit of the DNA-dependent RNA polymerase primarily due to missense mutations within codons of a highly conserved region of the *rpoB* gene, referred to as the rifampin resistance determining region (RRDR).\(^7,39,41,42\) Rifampin resistance in *M. leprae* also correlates with missense mutations within the RRDR of *rpoB*. Substitutions within codon Ser425 have been shown to be the most frequent mutations associated with the development of the rifampin resistant-phenotype in *M. leprae* (Table 1).

**CLOFAZIMINE**

Clofazimine [3-p-chloroanilino]-10-(p-chlorophenyl)-2,10-dihydro-2-(isopropylimino) phenazine] is a substituted imiphenazine with anti-mycobacterial activity whose mechanism of action has not been fully elucidated.\(^1,13,16,21\) The most important virtues of clofazimine with regard to anti-leprosy activity are its ability to attain high intracellular levels in mononuclear phagocytic cells, anti-inflammatory activity, low incidence of drug resistance, and slow metabolic elimination.\(^43\) It is highly lipophilic and appears to bind preferentially to mycobacterial DNA.\(^1\) Binding of the drug to DNA appears to occur principally at base sequences containing guanine, explaining clofazimine’s preference for the GC-rich genomes of mycobacteria, as compared with human DNA.

Recently, it was discovered that lysophospholipids mediate the activity of clofazimine in some gram-positive bacteria;\(^44\) however, it is unclear whether this mechanism of action is operational in *M. leprae*. Since it appears that clofazimine may act through many different mechanisms, it is not difficult to understand why only a few cases of clofazimine resistant leprosy have been reported over the years.\(^19,26,45,46\)

**MINOCYCLINE**

Minocycline (7-dimethylamino-6-demethyl-6-deoxytetracycline) is the only member of the tetracycline group of antibiotics to demonstrate significant activity against *M. leprae*, presumably due to its lipophilic property, which may enhance cell wall penetration.\(^1,22,23\) Minocycline is bactericidal for *M. leprae* and its activity is additive when combined with dapsone and rifampin.\(^22,23\) The mechanism of action of minocycline against *M. leprae* is unknown, but thought to be similar to that of all tetracyclines which act by inhibiting protein synthesis. Tetracyclines bind reversibly to the 30S ribosomal subunit, blocking the binding of aminoacyl transfer RNA to the messenger RNA ribosomal complex.\(^47\) Resistance to tetracycline may be mediated by one of three different mechanisms: 1) an energy-dependent efflux of tetracycline brought about by an integral membrane protein; 2) ribosomal protection by a soluble protein; or 3) enzymatic inactivation of tetracycline. The molecular mechanism of minocycline resistance has not been studied in *M. leprae* primarily because this drug has only recently been used for treating single lesion paucibacillary leprosy and resistant strains have not been identified.

**OFLOXACIN**

Ofloxacin (4-fluoroquinolone) is a fluorinated carboxyquinolone and demonstrates moderate antileprosy activity.\(^1,20–22\) The mechanism of action of ofloxacin on *M. leprae* is unknown
but in other bacteria it appears to inhibit DNA replication by inhibiting the DNA gyrase, a type II topoisomerase.\textsuperscript{48,49} The DNA gyrase in bacteria is a tetramer containing two A-subunits (GyrA) and two B-subunits (GyrB) encoded by gyrA and gyrB, respectively. The GyrA of \textit{M. leprae}, unlike its counterpart in \textit{M. tuberculosis} or \textit{M. smegmatis}, is produced by protein splicing by the excision of an intein, encoded by a 1260 bp in-frame insertion sequence, found in \textit{gyrA}.\textsuperscript{50} Mutations within a highly conserved region of \textit{gyrA}, the quinolone resistance determining region (QRDR), are associated with the development of ofloxacin resistance in most resistant strains of mycobacteria.\textsuperscript{49,51} The QRDR of \textit{M. leprae} \textit{gyrA} is highly homologous with that of \textit{M. tuberculosis} and missense mutations within this region have been found in ofloxacin-resistant strains of \textit{M. leprae}\textsuperscript{27,28} (Table 1).

**Development of drug resistance in \textit{M. leprae}**

Lacking direct evidence in \textit{M. leprae} for mechanisms of resistance of most of the antileprosy drugs, our current understanding is based on studies carried out in \textit{M. tuberculosis}, other bacteria and limited studies with \textit{M. leprae} genes in surrogate hosts. From these studies one can predict that: 1) drug resistance in \textit{M. leprae} is attributed to chromosomal mutations in genes encoding drug targets; 2) that these mutations occur spontaneously as a result of errors in DNA replication; and 3) these mutants are further enriched in a population by inappropriate or inadequate drug therapy.

Because \textit{M. leprae} cannot be cultivated in vitro, the frequency of drug resistant mutants in a population is primarily inferred from studies in \textit{M. tuberculosis}. For example, the frequency of dapsone-resistant mutants in a population of \textit{M. leprae} is estimated to be $10^{-6}$ and the frequency for rifampin or ofloxacin resistance is estimated at $10^{-7}$ to $10^{-8}$.\textsuperscript{41} Rates of clofazimine resistance in \textit{M. leprae} are unknown, but appear to be relatively low. Since untreated multibacillary patients can harbour large bacterial loads ($>10^{11}$ \textit{M. leprae}), it is feasible that a patient could contain up to $10^5$ dapsone-resistant organisms and thousands of rifampin- or ofloxacin-resistant organisms. Inappropriate therapy (non-compliance or inadequate therapy) for these patients has the potential to enrich sub-populations of drug-resistant \textit{M. leprae}, thereby, leading to the spread of one or more resistant phenotypes. Indeed, drug-resistant isolates of \textit{M. leprae} have been found in many parts of the world.\textsuperscript{19,26–28,52,53}

**Detection of drug resistance in leprosy**

Conventional drug susceptibility testing of \textit{M. leprae} from clinical specimens relies on the ability to cultivate \textit{M. leprae} in the hind footpads of mice according to the method described by Shepard.\textsuperscript{54} This method requires the recovery of a sufficient number of viable organisms from a patient to inoculate the foot pads of 20–40 mice (depending on the number of drugs to be tested) with each foot pad receiving $5 \times 10^3$ organisms. Results are available after 6 months to 1 year. Because of the need for certain numbers of bacteria, only patients with moderate to large bacterial loads can be tested. The first rapid drug screening assays for \textit{M. leprae} were developed based on radiorespirometric techniques (BACTEC or Buddemeyer) and have been used successfully to identify new anti-leprosy drugs.\textsuperscript{55} While these techniques have shown wide applicability for drug screening, their use for drug susceptibility testing in leprosy is limited by a stringent requirement for large numbers ($\geq 10^7$) of viable organisms from each patient.
Detection of drug resistant *M. leprae*

![Figure 1](image)

**Figure 1.** Detection of rifampin resistance in *Mycobacterium leprae* using PCR-direct DNA sequencing. Bottom panel shows the partial *rpoB* RRDR DNA sequence of rifampin-susceptible (RMP\(^s\)) *M. leprae*; top panel shows the partial *rpoB* RRDR DNA sequence of Ser425Leu (TCG-TTG) rifampin resistant (RMP\(^r\)) *M. leprae*.

In light of continued reports of drug-resistant leprosy in various parts of the world, molecular assays for resistance would simplify susceptibility testing and provide a means for monitoring resistance globally. To reduce the number of organisms needed and to minimize the time required for drug susceptibility testing of *M. leprae*, several protocols based on genotypic identification of drug-resistant mutants have been developed. These techniques are based on the amplification of specific DNA fragments from crude biological specimens (e.g. skin biopsy specimens from leprosy patients) using polymerase chain reaction (PCR) amplification and detection of mutations associated with drug resistance within these DNA fragments.

PCR is a procedure for enzymatically producing large amounts of DNA of defined length and sequence from small quantities of DNA present in crude biological specimens. This technique mimics the natural replication of chromosomal DNA using simple equipment and reagents. PCR has created new approaches for detecting and identifying *M. leprae*\(^{56,57}\) and, coupled with mutation detection analyses, has the capability of providing rapid drug susceptibility results from specimens taken directly from the patient. PCR-based mutation detection assays that have been developed for drug susceptibility testing of *M. leprae* in clinical specimens include: PCR-direct DNA sequencing, PCR-single-strand conformation polymorphism analysis (PCR-SSCP), PCR-heteroduplex analysis (PCR-HDA), and PCR-solid phase reverse-hybridization analysis.

**PCR-DIRECT DNA SEQUENCING**

Direct DNA sequencing for drug resistance is the most definitive of all of the nucleic acid-
based mutation detection protocols because it detects the actual nucleotide changes in the target gene in which mutations associated with antibiotic resistance are found. In addition, the assay can be designed to be species-specific, providing direct evidence for the presence of a particular pathogen in the specimen being tested. PCR-direct DNA sequencing has been used to identify rifampin-, dapsone-, and ofloxacin-resistant mutants of *M. leprae* (Table 1). These assays are based on PCR amplification of the appropriate target DNA directly from skin biopsy specimens using oligonucleotide primers that are specific for the RRDR, SRDR, or the QRDR of *M. leprae*. The DNA sequence of these PCR products is then obtained by either automated or manual dideoxy DNA sequencing with primers flanking these regions. The resultant DNA sequence is then examined for the presence of mutations previously associated with drug resistance in *M. leprae*. An example of the results obtained by PCR-direct DNA sequencing for rifampin-susceptible and resistant mutants of *M. leprae* is shown in Figure 1. The mutation detected (TCG?TTG) results in the substitution of a leucine amino acid residue for a serine in codon 425 of the β-subunit of the RNA polymerase. This is the most frequently detected mutation associated with rifampin resistance in *M. leprae*.

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**Figure 2.** Detection of rifampin resistance in *Mycobacterium leprae* using the PCR-single strand conformation polymorphism (PCR-SSCP) assay. S corresponds to the SSCP pattern of the denatured rpoB RRDR DNA fragment of a rifampin-susceptible (RMP5) *M. leprae*. R corresponds to the SSCP pattern of the denatured rpoB RRDR DNA fragment of a rifampin-resistant (RMP6) *M. leprae*.

**Figure 3.** PCR-solid phase reverse hybridization assay for detection of rifampin resistance of *Mycobacterium leprae*. **A** Map of 83-bp RRDR of *M. leprae* rpoB and hybridization capture probes. Capture probes that bind native rpoB sequence are labelled 1–4, A27 and A34–39. Capture probes that bind specific mutations in this region are labelled A30R1 that detects TTG (Ser425Leu) mutation, A32R3 that detects ATG (Ser425Met) mutation and A33R9 that detects TTC (Ser425Phe) mutation, associated with the development of rifampin resistance in *M. leprae*. **B** Detection of mutations associated with rifampin resistance in *M. leprae* using PCR-solid phase reverse hybridization analysis: lane 1, hybridization profile produced with a rifampin-susceptible *M. leprae* containing wild-type rpoB RRDR; lanes 2, 3 and 4, hybridization profiles produced from plasmid templates containing the RRDR of *M. leprae* with either a TTG, ATG or TTC mutation, respectively, in codon Ser425; lane 5, hybridization profile of a rifampin-resistant strain of *M. leprae* containing the RRDR with a GAC mutation in codon 420, resulting in the substitution of a aspartic acid residue for a histidine residue. Figure was reproduced with permission from Nadine Honore.
Detection of drug resistant *M. leprae*

(Table 1). PCR-direct DNA sequencing can be performed in a well-equipped diagnostic laboratory with either manual or automated DNA sequencing systems and requires approximately 1–2 days to obtain drug-susceptibility results directly from clinical specimens.
PCR-SSCP

Honore et al. developed a PCR-SSCP assay to detect rifampin-resistant *M. leprae* from human specimens. To accomplish this, the RRDR target was amplified by PCR. These double-stranded PCR products were then dissociated into single strands using heat and then separated by denaturing gel electrophoresis under stringently controlled temperature conditions (Figure 2). Gels were then stained to observe DNA fragment mobility patterns called SSCP profiles. A schematic of SSCP patterns obtained with rifampin-resistant and susceptible mutants of *M. leprae* is shown in Figure 2. DNA strands from a rifampin-susceptible organism (S) migrate at a rate proportional to their molecular weight and conformation and give a reproducible SSCP profile (Figure 2, lane 1). Strands from a rifampin resistant organism (R) containing a Ser425-Leu (TCGTTG) mutation, demonstrate a unique mobility pattern, reflecting the change in nucleotide composition (Figure 2, lane 2). The DNA fragment patterns observed with SSCP are highly reproducible and yield profiles unique to specific mutations.

PCR-SOLID PHASE REVERSE HYBRIDIZATION ANALYSIS

The solid phase reverse hybridization assay for detection of rifampin resistance in *M. leprae* is similar to the line probe assay (LiPA) used for detection of rifampin resistance in M. tuberculosis. An initial PCR step with a biotinylated and an unlabelled primer produces an 83-bp, biotinylated fragment of the *M. leprae* RRDR (Figure 3A). The amplified PCR product is then hybridized to a set of DNA capture probes, which have been immobilized at specific sites on a Biodyne C membrane. The immobilized capture probes are small DNA fragments that are either homologous with short segments of the RRDR of rpoB from a rifampin-susceptible strain of *M. leprae* (1–4 and A27, A34–A39) or specific mutant strains (A30R1, A32R3, A33R9 (Figure 3A). The stringency of the hybridization reaction is designed so that the PCR product will only bind to probes with 100% sequence homology. The resultant hybrids are detected by chemiluminescence using a streptavidin peroxidase conjugate. The genotype of the test organism is determined by which capture probes produce hybridization signals. In Figure 3B, lane 1 represents a rifampin-susceptible strain because all lines containing the wild-type DNA sequence (1–4, 27A, 34A–39A) produced positive hybridization signals. The hybridization profiles seen in lanes 2, 3 and 4 were produced from plasmid templates encoding the RRDR of *M. leprae* with a TTG, ATG or TTC mutation within codon Ser425. Lane 5 shows the result when a rifampin-resistant strain of *M. leprae* possesses the His420Asp mutation as a result of the missense mutation GAC.

PCR-HETERODUPLEX DETECTION

PCR-heteroduplex detection of drug resistance in bacteria was initially developed to detect the presence of drug-resistant *M. tuberculosis* from sputum specimens using a universal heteroduplex generator (UHG). A similar approach has been used to develop a PCR-UHG assay to detect the presence of dapsone-resistant *M. leprae* from skin biopsy homogenates of lepromatous leprosy patients (Figure 4). The assay requires PCR amplification of the SRDR of *folP1* and the mixing of these PCR products with a universal heteroduplex generator (UHG-DDS-141). The UHG-DDS-141 is a synthetic 141 bp SRDR DNA fragment that contains several base pair mismatches flanking *folP1* codons 53 and 55 (mutations spontaneously occur within these codons that are associated with dapsone resistance).
Figure 4. Detection of dapsone resistance in *Mycobacterium leprae* using the PCR-heteroduplex (PCR-UHG) assay. A Schematic of the PCR-UHG procedure; B ethidium bromide-stained 6% Tris borate EDTA (TBE) PAGE gel containing heteroduplex patterns from dapsone-resistant (R) and dapsone-susceptible (S) *M. leprae* strains; (−) contains UHG-DDS-141 and an equivalent volume of PCR control buffer in the heteroduplex reaction. 231 bp and 144 bp fragments represent the homoduplexes.
When the UHG-DDS-141 is denatured by heat and slowly annealed to denatured SRDR PCR products from \textit{M. leprae}, the resultant heteroduplexes form unique structures which, when analysed by electrophoresis, provide enhanced mutation detection over standard heteroduplex detection. Enhanced mutation detection occurs because large areas of unmatched nucleotides (bubbles) in the newly formed duplexes greatly affect the mobility of the resultant DNA fragments (Figure 4A). Therefore, when the heteroduplexes are separated by gel electrophoresis on polyacrylamide minigels and stained with ethidium bromide, unique heteroduplex profiles are observed for susceptible and resistant genotypes (Figure 4B). The PCR-UHG requires approximately 6 h to complete, uses 6% precast non-denaturing Tris-borate-EDTA minigels and a non-radioactive detection format.

**Future perspectives for drug susceptibility testing in leprosy**

The methodology for drug susceptibility testing in leprosy has not improved in over 30 years. Whereas the mouse footpad assay for drug susceptibility testing requires low-technology resources, the turn-around time to obtain results is unacceptable for clinically meaningful drug resistance testing. In contrast, molecular assays designed to detect drug-resistant \textit{M. leprae} are configured to yield results within a few days using standard technology available in most modern microbiology laboratories. DNA-based assays can detect mutations in the genome of \textit{M. leprae} directly from clinical specimens without the need to expand the bacteria in mice. Unfortunately, not all mutations yielding drug resistance in \textit{M. leprae} have been identified, nor has each mutation found in various drug-target genes been validated, leaving the molecular approach unfinished. The major limitations to expanding our knowledge of mechanisms of drug resistance are our inability to cultivate \textit{M. leprae} on axenic medium and to perform genetic studies to validate the relationship between mutations and their association with drug resistance. Accordingly, more research is needed to increase our knowledge of resistance mechanisms in \textit{M. leprae}, and thereby further authenticate target gene-based molecular assays.

In this review, we have described the molecular aspects for the mode of action of, and the mechanism of resistance to, the major drugs used to treat leprosy. We have attempted to relate how these findings have been or could be used to develop molecular assays for rapid detection of drug resistant \textit{M. leprae}. Currently the number of reports describing clinical relapses due to single or multiple-drug-resistant \textit{M. leprae} is low. This may reflect the reality of the problem or possibly be the result of a virtually nonexistent post-MDT surveillance program for relapse. Establishing regional and global monitoring of drug resistance in leprosy is an important goal to pursue and in so doing may help define the dynamics of drug resistance in leprosy. As newer molecular tests for detecting drug-resistant \textit{M. leprae} are implemented, a fuller view of potential risks involved in the current strategy to eliminate leprosy, based on MDT, can be evaluated. Finally, the achievement reached with the development of rapid and reliable molecular assays for detecting rifampin, dapsone and ofloxacin resistance in leprosy provides hope that new molecular assays can be developed for detecting resistance to other drugs used to treat leprosy.

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References


