Leprosy Drug Resistance Surveillance in Colombia: The Experience of a Sentinel Country

Camilo Beltrán-Alzate¹, Fernando López Díaz², Marcela Romero-Montoya¹, Rama Sakamuri³, Wei Li³, Miyako Kimura³, Patrick Brennan³, Nora Cardona-Castro¹,⁴*

¹ Instituto Colombiano de Medicina Tropical–Universidad CES Sabaneta, Antioquia, Colombia, ² Sanatorio de Agua de Dios, Agua de Dios Cundinamarca, Colombia, ³ Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, Colorado, United States of America, ⁴ Facultad de Medicina. Universidad CES, Medellín, Colombia

* ncardona@ces.edu.co

Abstract

An active search for *Mycobacterium leprae* drug resistance was carried out, 243 multibacillary patients from endemic regions of Colombia were included from 2004 to 2013 in a surveillance program. This program was a World Health Organization initiative for drug resistance surveillance in leprosy, where Colombia is a sentinel country. *M. leprae* DNA from slit skin smear and/or skin biopsy samples was amplified and sequenced to identify mutations in the drug resistance determining region (DRDR) in *rpoB*, *folP1*, *gyrA*, and *gyrB*, the genes responsible for rifampicin, dapsone and ofloxacin drug-resistance, respectively. Three isolates exhibited mutations in the DRDR *rpoB* gene (Asp441Tyr, Ser456Leu, Ser458Met), two in the DRDR *folP1* gene (Thr53Ala, Pro55Leu), and one isolate exhibited mutations in both DRDR *rpoB* (Ser456Met) and DRDR *folP1* (Pro55Leu), suggesting multidrug resistance. One isolate had a double mutation in *folP1* (Thr53Ala and Thr88Pro). Also, we detected mutations outside of DRDR that required in vivo evaluation of their association or not with drug resistance: *rpoB* Arg505Trp, *folP1* Asp91His, Arg94Trp, and Thr88Pro, and *gyrA* Ala107Leu. Seventy percent of *M. leprae* mutations were related to drug resistance and were isolated from relapsed patients; the likelihood of relapse was significantly associated with the presence of confirmed resistance mutations (OR range 20.1–88.7, p < 0.05). Five of these relapsed patients received dapsone monotherapy as a primary treatment. In summary, the current study calls attention to *M. leprae* resistance in Colombia, especially the significant association between confirmed resistance mutations and relapse in leprosy patients. A high frequency of DRDR mutations for rifampicin was seen in a region where dapsone monotherapy was used extensively.
Author Summary

*Mycobacterium leprae* drug resistance is cause of surveillance due to the increase of leprosy relapsed cases. World Health Organization initiative for drug resistance surveillance in leprosy included Colombia, a country considered in post-elimination stage, as a sentinel country. During 10 years (2004–20013) an active search for *M. leprae* drug resistance was carried out in volunteer patients (each of whom signed a consent form) recruited from a convenience sample of patients diagnosed with leprosy in fourteen departments of Colombia: Amazonas, Antioquia, Atlántico, Bolívar, Caquetá, Cesar, Cundinamarca, Chocó, Huila, Magdalena, Norte de Santander, Santander, Tolima, and Valle. 243 multibacillary patients in various stages of multi drug therapy (MDT) were enrolled with the aim to search for primary and secondary *M. leprae* drug resistance: 33 new patients before treatment, 136 currently undergoing MDT with not clinical improvement of lesions after three months or more of MDT, 36 post-MDT with positive bacillary index (BI) persistence, 4 non-adherent to MDT, and 34 relapsed cases. *M. leprae* DNA from patient’s samples was tested to identify mutations in the drug resistance determining region (DRDR) in *rpoB*, *folP1*, *gyrA*, and *gyrB*, the genes responsible for rifampicin, dapsone and ofloxacin drug-resistance, respectively. We obtained isolates that exhibited mutations in the DRDR, three in *rpoB* gene, two in *folP1* gene, and one isolate exhibited mutations in both *pboB* and *folP1*, suggesting multidrug resistance. One isolate had a double mutation in *folP1*. Also, we detected mutations outside of DRDR that required in vivo test validation. 70% of *M. leprae* confirmed resistance mutations were isolated from relapsed patients (OR range 20.1–88.7, p < 0.05). Five of these relapsed patients received dapsone monotherapy as a primary treatment. The current study calls attention to *M. leprae* resistance in Colombia, especially the significant association between confirmed resistance mutations and relapsed in leprosy patients.

Introduction

Leprosy is a chronic infectious disease caused by *M. leprae* that results in disfigurement, exclusion from society and, often, in or from poverty [1]. Prior to the 1940s, leprosy was considered an untreatable disease. However, in the early 1940s, diamino-diphenyl sulfone (DDS; also known as dapsone), was shown to successfully treat leprosy. At that time, dapsone was used for treatment of leprosy until lesions were cured or until the bacillary index (BI) became negative, a process that took decades [2, 3] and one that often resulted in patients relapsing after the termination of treatment [3]. In the 1980’s, the World Health Organization (WHO) recommended dapsone, rifampicin and clofazimine as the primary agents for multidrug therapy [4]. The frequency of clinical relapse due to dapsone resistance after monotherapy, is higher than the results of dapsone resistance tests in mouse foot pad since few strains exhibited full resistance [5]. Nevertheless, resistance to low levels of dapsone is detected in mouse footpads, indicative of inadequate treatment. Dapsone is a bacteriostatic antibiotic and the persistence of bacilli may be one cause of relapse in dapsone monotherapy [6].

WHO, concerned with clinical relapses, emergence, and transmission of organisms with low levels of resistance to dapsone, recommended the addition of rifampicin, a bactericidal drug taken from the TB treatment stock [4]. Thus, multidrug therapy (MDT) for leprosy was initiated in the early 1980s [3,4]. To treat paucibacillary leprosy (PB), the MDT combination is dapsone and rifampicin. To treat multibacillary (MB) leprosy, clofazimine was included as a third drug [4,6]. Currently, WHO recommends an MDT duration of 1 year for MB leprosy and
6 months for PB leprosy. Globally, these guidelines apply to more than a million patients, having been adapted to a variety of resource-constrained settings over the past decades [3].

In Colombia, MDT was introduced in 1985 when the prevalence of leprosy was 5.5 cases per 10,000 people. In 1997, Colombia achieved the goal of elimination proposed by WHO: a prevalence of less than 1/10,000. Although leprosy treatment in Colombia is based on WHO guidelines, some modifications are made according to the criteria of clinicians [7,8]. For example, the duration of treatment for PB patients may be extended for one year, and in the case of MB the treatment, up to 2 years, if lesions persist and the Bacillary index (BI) is positive [7,9]. Leprosy resistance in Colombia has been associated with relapses, especially in patients undergoing dapsone monotherapy [10, 11].

When the molecular targets for DDS and rifampicin were identified, drug resistant bacterial strains with high resistance in mouse footpad systems were found to have mutations in genes encoding dihydropteroate synthase (dhps or folP1) and RNA polymerase B (rpoB) [12–15]. However, drug resistant strains exist that do not have mutations in known genetic targets [3, 4]. While mutations that confer low levels of resistance have not been identified, such low-level resistance is seen in wild-type strains [4, 13]. The mechanism of clofazimine resistance has been under investigation for many years. Recently, it was shown that clofazimine is a substrate for bacterial type-2 NADH reductase [16]. However, mutations for genes involved in this redox process have not been ascribed to clinical clofazimine resistance [16]. MDT treatment of new leprosy cases is not routinely accompanied by monitoring for primary or secondary drug resistance [3, 6]; surveillance and retreatment of dapsone monotherapy in patients undergoing MDT is practiced in only a few settings [15,17]. Treatment of returning patients with additional rounds of MDT, without knowledge of eventual underlying drug resistance, is the default course of action in leprosy control programs [7,18,19]. Unfortunately, dwindling resources for leprosy does not allow for individualized treatment regimens and long-term follow-ups [7]. Research has shown that patients with a high pre-MDT bacteriological index (>4) are at risk of relapsing after MDT, with relapses occurring 6–16 years after the completion of treatment [12]. An earlier study showed that a high pre-MDT bacterial index is also a risk factor for relapse. Relapses were not due to drug resistance, as partial and full dapsone resistance was detected in only 3 of 18 relapses [20].

Thus, distinguishing relapses due to incomplete treatment, persistent bacilli, drug resistance and re-infection are challenges for clinicians and health care workers. The definition of relapse differs for those undergoing dapsone monotherapy and those receiving fixed, 2 year MDT (and the now shortened 1 year MDT) [19]. Reduction of BI is a slow process, dropping only one unit each year with MDT. Therefore, no proper end point for the completion of treatment exists and BI determination is not a universally practiced standard of care [19].

For better understanding of the extent of primary and secondary drug resistance, researchers worldwide have independently used molecular epidemiological approaches. In 2006, WHO called for leprosy drug resistance surveillance (DRS) in several sentinel countries [18, 19]. This goal of this study was to report the DRDR mutations in leprosy patients from Colombia from a 10 year WHO recommended surveillance program.

**Methods**

**Patients**

This molecular epidemiology survey was performed using 243 individual *M. leprae* DNA samples from Colombian leprosy patients from 2004 to 2013. Volunteer patients (each of whom signed a consent form) were recruited from a convenience sample of patients diagnosed with leprosy in fourteen departments of Colombia: Amazonas, Antioquia, Atlántico, Bolívar,
Caquetá, Cesar, Chocó, Cundinamarca, Huila, Magdalena, Norte de Santander, Santander, Tolima and Valle. Patients, in various stages of treatment were enrolled with the aim to search for primary and secondary *M. leprae* drug resistance and included 33 new patients before treatment, 136 currently undergoing treatment with no clinical improvement of lesions after three months of treatment, 36 post-treatment with positive bacillary index (BI) persistence, 4 non-adherent to treatment, and 34 relapse cases.

Bacterial DNA was extracted from skin biopsies using the Dneasy Blood & Tissue Qiagen kit (Invitrogen) according to the manufacturer’s protocol [21,22]. Slit skin samples were collected from four different sites, with an emphasis on active lesions. Extracted DNA was stored at -20°C until processing.

**Ethical considerations**

The ethical committee of the Instituto Colombiano de Medicina Tropical–Universidad CES approved this project as the minimal risk, all the data analyzed were anonymized.

**Amplification and sequencing analysis**

Multiplex PCR was performed using primers suggested by WHO to amplify the DRDR regions of the *rpoB*, *folP1*, *gyrA* and *gyrB* genes [19]. PCR reactions were performed in 25 μl reaction volumes: 2.50 μl of 10X AmpliTaq Gold PCR Mastermix (Applied Biosystems, USA), 2.50 μl of MgCl2 (25 mM), 1.25 μl of each primer (0.3 mM), 8μl of sterile, deionized water and 2.0 μl extracted DNA. A PIKO thermocycler (Finnzymes Instruments) was employed with the following amplification conditions: activation of AmpliTaq Gold at 95°C for 5 min, 40 cycles at 95°C for 15 sec, 60°C for 15 sec, 72°C for 60 sec, and a final elongation at 72°C for 7 min.

For each amplification, 2 μl of purified *M. leprae* DNA (reference strain 4264) was used as a positive control. 2 μl of RNase-free ultrapure water and 2 μl of DNA of *Salmonella enteritidis* (ICMT-99 strain) were each used as negative controls. DNA products were electrophoresed on agarose gel in 2.5% agarose gel in TE buffer and stained with ethidium bromide. A 20 pb molecular weight marker was used to determine amplicons size (Biorad, Cat No. 1708351).

PCR products were sequenced using on an ABI PRISM 3130xl genetic analyzer (Applied Bio systems) at the Colorado State University core sequencing facility. Chromatograms were analyzed with Chromas lite software [23] and compared with *M. leprae* reference sequences of antibiotic sensitive strains (in the leproma database [http://genolist.pasteur.fr/Leproma/]) by BLAST, as suggested by WHO [24].

**Statistical analysis**

The odds ratio and 95% CI of the relationship between likelihood of relapse and the presence of confirmed resistance mutations was calculated and significance assessed by Chi square test. \( P < 0.05 \) was considered significant.

**Results**

The geographical distribution of patients included in this study and the origin of *M. leprae* DRDR mutations are shown in Fig 1. The Andean region had 50% of *M. leprae* DRDR mutations. Of note, within this Andean region are the departments of Cundinamarca and Santander where two existing Colombian leprosaria, Agua de Dios and Contratación, are located.

The distribution of samples according to patient origin, gender and age is summarized in Table 1. Overall, the male to female ratio was 2.45:1; 72.2% of patients were male. The mean age was 52 years with a range of 9 to 91 years.
Fig 1. Geographical origin of leprosy patients that exhibits *M. leprae* drug resistance related mutations.

doi:10.1371/journal.pntd.0005041.g001
Positive PCR and successful sequencing were not obtained in all cases and could be due to DNA as a consequence of treatment. Positive PCR and successful DNA amplification were exhibited for \textit{rpoB} (n = 134/243), \textit{folP1} (n = 215/243), \textit{gyrA} (n = 200/243) and \textit{gyrB} (n = 184/243).

Sequences showed that three isolates exhibited mutations in DRDR \textit{rpoB} gene (Asp441Tyr, Ser456Leu, Ser458Met), and two in DRDR \textit{folP1} gene (Thr53Ala, Pro55Leu). One isolate exhibited mutations in both DRDR \textit{rpoB} (Ser456Met) and DRDR \textit{folP1} (Pro55Leu). This result in this isolate suggested multidrug resistance (Table 2).

One isolate had a double mutation in \textit{folP1}: Thr53Ala and Thr88Pro (outside of DRDR). Six mutations outside of DRDR that required an in vivo test for validation were found: \textit{rpoB} Ala426Thr and Arg505Trp, \textit{folP1} Asp91His, Arg94Trp, and Thr88Pro, and \textit{gyrA} Ala107Leu (Table 2).

It is remarkable that all mutations found in DRDR for \textit{rpoB} and \textit{folP1} were detected in seven relapsed patients and in one patient currently undergoing treatment (Table 2).

Mutations found in \textit{rpoB} (Ala426Thr, Asp441Tyr, Ser456Leu, Ser458Met, and Arg505Trp) were within, or close to, the DRDR found between nucleotides 432 to 458 [25–29]. The \textit{folP1} mutations (Thr53Ala and Pro55Leu) were located in the DRDR [28, 30].

Further, we found isolates from three relapsed patients that exhibited mutations that will require further validation by mouse footpad method, in specialized laboratories—two isolates in \textit{rpoB} and one isolate in \textit{folP1}. The two mutations in \textit{rpoB} (Ala426Thr and Arg505Trp) were close to the rifampicin resistance determining region located between nucleotides 432 and 458.
The \( \text{folP1} \) mutation (Asp91His) was located outside the dapsone resistance determining region, at positions 53 and 55 [28, 30].

Regarding \( \text{gyrA} \), no isolate displayed a mutation in the region determining fluoroquinolone resistance (nucleotides 89–91) [28–31]. However, one patient under treatment exhibited a mutation within \( \text{gyrA} \) (Ala107Leu) that also will require further validation by mouse footpad method, in specialized laboratories, for ofloxacin resistance.

In terms of frequency, five of the 134 cases with a positive amplification and DNA sequence (3.7%) had mutations in \( \text{rpoB} \), five of 215 (2.32%) in \( \text{folP1} \), and one of the 200 (0.5%) in \( \text{gyrA} \). None contained a mutation in \( \text{gyrB} \).

Four of 34 (11.8%) relapsed patients exhibited isolates with mutations in the DRDR \( \text{rpoB} \) and/or \( \text{folP1} \). In contrast, one of 209 (0.47%) non-relapse patients contained isolates with mutations in DRDR \( \text{folP1} \)mutations.

### Table 2. Clinical characteristics of patients exhibited \( \text{M. leprae} \) DRDR mutations.

| Date of report/Code* | Age | Sex | Lesions | Number of lesions | Bacillary Index | Mutations | Date Past Diagnosis/Therapy | Actual Diagnosis/Therapy |
|----------------------|-----|-----|---------|------------------|----------------|-----------|----------------------------|--------------------------|
| 2006/ Code 1         | 66  | M   | Lepromas in arms and legs | 15              | 3+            | \( \text{rpoB Asp441Tyr} \)+ | 1960-LL/MNT**            | Relapse LL/MDT           |
| 2008/ Code 2         | 50  | M   | Nodules, skin infiltrates in face, thorax, abdomen, legs | 20              | 1+            | \( \text{folP1 Thr653Ala} + \text{folP1 Thr88Pro} \)+ | 1976-LL/MNT              | Relapse LL/MDT           |
| 2010/ Code 3         | 66  | M   | Type II reaction, anesthesia, paresthesia, hypo and hyper pigmented plaques in legs and arms, thorax, back,leonine fascies, madarosis, nodules, infiltrates | >25             | 2+            | \( \text{rpoB Arg505Trp} + \) | 1990-LL/MDT***          | Relapse LL/MDT           |
| 2006/ Code 4         | 58  | M   | Nodules in arms, legs, loss of sensitivity | 7               | 1+            | \( \text{rpoB Ser458Me} + \text{folP1 Pro55Leu} + \) | 1972-LL/MNT 1983-LL/MDT 2003-Leprosy Reaction 2006-LL/MDT | Relapse LL/MDT           |
| 2008/ Code 5         | 42  | M   | Hypo and hyper-pigmented macula, skin infiltrates in arms, thorax, abdomen, legs since two years ago. | 12              | 3+            | \( \text{folP1 Thr53Ala} + \) | Under treatment LL/MDT  | |
| 2006/ Code 6         | 52  | M   | Nasal septum affected, claw hands, ulnar nerve thick, skin patches, infiltration in arms, legs, since six months | 10              | 2+            | \( \text{folP1 Asp91Hist} \)† | 1967-LL/MNT 1988-LL/MDT | Relapse LL/MDT           |
| 2011/ Code 7         | 81  | M   | Lesions, macules in superior limbs, back, thorax, generalized xerosis, bone reabsorption in right foot, madarosis, tibial ulcers | 11              | 2+            | \( \text{rpoB Ala426Thr} \)† | 1990-LL/MDT | Relapse LL/MDT           |
| 2008/ Code 8         | 66  | M   | Macular lesions in ear lobes | 2               | 1+            | \( \text{gyrA Ala107Leu} \)† | Under treatment BL/MDT  | |
| 2011/ Code 9         | 84  | M   | Macules, plaques | >5              | 5+            | \( \text{rpoB Ser456Leu} + \) | 1946-LL/MNT2011-LL/MDT  | Relapse or Reinfection not confirmed/ MDT |
| 2011/ Code 10        | 56  | M   | Leprosy reaction | >5              | 2+            | \( \text{folP1 Arg94Trp} \)† | 2010-LL/MDT | Leprosy reaction/MDT     |

* Code of patients exhibiting \( \text{M. leprae} \) drug-resistance mutations is located in Fig 1.

** MNT monotherapy DDS.

*** Multidrug therapy.

+ DRDR: mutation inside Drug Resistance Determining Region.

† Mutation outside DRDR needs confirmation by foot pad mice test.

doi:10.1371/journal.pntd.0005041.t002
Overall, the likelihood of relapse was significantly associated with the presence of confirmed resistance mutations: resistance to dapsone (OR 20.1 [95% CI 2.0–99.6], p = 0.01), resistance to rifampicin (OR 39.7 [95% CI 2.1–56.9], p = 0.01), and resistance to either dapsone or rifampicin (OR 88.7 [95% CI 10.5–747.3], p < 0.01).

Discussion

In this report, drug resistant *M. leprae* strains from 243 patients at various leprosy clinical and treatment stages were determined. The study utilized a patient group assembled over a ten-year period. While the threshold for alarm over drug resistance to MDT, locally and globally, has not been well defined, we demonstrate that single and multi-drug resistant strains are detectable in Colombia, albeit at low frequencies [10, 11].

Of notable concern is the detection of a folP1 mutation previously related with drug resistance in one patient under MDT that had not previously undergone DDS treatment, and in two others completing one and two treatments of MDT. Similarly, three patients had rpoB drug resistance mutations—one exposed to rifampicin during the first cycle of MDT and two treated with more than one cycle of MDT. The finding that one patient had dapsone and rifampicin resistance, combined with a previous report concerning two cases of rifampicin resistance [10], suggests that the rifampicin resistance is not trivial in Colombia, a country considered in the post-elimination stage of leprosy (prevalence <1/10,000). Despite the known presence of dapsone resistance mutations, relapsed patients are commonly retreated with an MDT regimen that contains dapsone.

We observed novel mutations in relapsed patients (Table 2) including folP1 Thr88Pro, rpoB Ala426Thr, Arg505Trp, and gyrA Ala107Leu. Clinical evidence of relapse and mutations DRDR genes, can help the clinicians to choose alternative treatments to the patient, such as a combination of rifampicin, ofloxacin and minocycline [32]. However, identifying drug resistance in leprosy patients implies clinical, logistical and financial challenges. Further, the change of treatment is not a common clinical practice since drug resistance tests are not available in the routine activities of the leprosy control programs in Colombia, much less in the majority of endemic regions [7]. Also, alternative treatments are not freely available in the leprosy control programs [7]. The results of this policy are evidenced in the seven cases we reported in this study which received regular MDT after relapse.

We found that Colombian dapsone monotherapy patients can relapse. Further, in patients carrying *M. leprae* drug resistant mutations, current results show an association between drug resistance mutations and relapse (OR range 20.1–88.7, p < 0.05). This result suggests that dapsone monotherapy patients require focused attention because they have the potential to act as a reservoir for dapsone resistant strains [10, 11, 33]. However, it is difficult to identify at-risk individuals for relapse. For example, patients associated with leprosaria have been found to have higher frequency of dapsone resistance in this and prior studies, similar to that reported in Brazil [32,33]. However, no single department or town was a leprosy hot spot in this cross sectional surveillance.

Mutations outside of the genetic regions associated DR had been described in other reports [34, 35]. Uncharacterized polymorphisms and novel mutations have to be tested in vivo to test for resistance and add to our knowledge base. Phenotypic tests for drug resistance, such as those reported by Nakata et al [36], could be developed. Perhaps a saturated mutational analysis of target genes could be attempted to distinguish neutral polymorphisms from mutations leading to drug resistance [37,38].

Other targets for DR had been described, Monot et al [39] described rpoT for rifampicin, Singh et al [34] reported not clear explanation about rifampicin resistance phenotype of
Airaku-3, a multi-drug resistance strain, the authors found two non-synonymous SNPs in transporter genes, \textit{ctpC} and \textit{ctpI}, however, a functional assay is required to determine if these variants confers any degree of rifampicin resistance.

This study had limitations and strengths. Due to sampling restrictions, conclusions regarding primary versus acquired resistance, and distinguishing relapse from reinfection, was not possible in the present study.

Current results are related with the initiative that WHO has formalized, an annual meeting for regions/countries serving as voluntary sentinel sites for leprosy drug resistance surveillance (DRS). The program has grown from its original focus on rifampicin resistance to molecular surveillance for dapsone and quinolone resistance [18]. The gene targets for quinolones are \textit{gyrA} and \textit{gyrB}, which encode \textit{gyrAses} A and B that are responsible for uncoiling DNA [37,40]. Reasons for including these targets are that ofloxacin is a standby drug for some patients and an alternative to the ROM (rifampicin, ofloxacin and minocycline) regimen. Moreover, fluoroquinolones are secondary drugs for TB [41]. An increase in availability and exposure to these drugs should be monitored for their potential to aid in the increase of drug resistant \textit{M. leprae} [41,42].

The current study calls attention to \textit{M. leprae} resistance in Colombia, especially the significant association between confirmed resistance mutations and relapse in leprosy patients. 70% of mutations we detected were related to drug resistance and were isolated from relapsed patients, five of which received dapsone monotherapy as a primary treatment. A high frequency of DRDR mutations for rifampicin was seen in a region where dapsone monotherapy was used extensively. The reported drug-resistance \textit{M. leprae} suggests that the situation needs to be monitored in Colombia and the sentinel surveillance program have to continue in Colombia [32].

**Acknowledgments**

The authors want to thank Dr. Varalakshmi Vissa, Dr. Mary Sanders, Margarita Rosa Giraldo Cifuentes (Coordinator of Leprosy Program Antioquia), Saray Agamez Correa (Coordinator of Leprosy Program Cesar), Decdy Magnolia González Rugeles (Coordinator of Leprosy Program Cundinamarca), Luz Dally Falla Puentes (Coordinator Leprosy Program Huila) Matilde Llanos Campos (Coordinator Leprosy Program Norte de Santander), Débora Villa Villa (Coordinator Leprosy Program Santander), Elvira Pretel de Manotas (Coordinator Leprosy Program Atlántico), Sara Luz Molina Vizcaíno (Coordinator Leprosy Program Bolívar), and Liliana Forero Zapata (Coordinator Leprosy Program Valle del Cauca) for helpful discussion and advice on this study.

**Author Contributions**

- **Conceptualization:** NCC PB.
- **Data curation:** CBA MRM NCC.
- **Formal analysis:** NCC CBA PB.
- **Funding acquisition:** NCC PB.
- **Investigation:** CBB MRM FLD RS WL MK NCC.
- **Methodology:** RS WL MK CBA MRM NCC.
- **Project administration:** NCC.
- **Resources:** NCC.
Supervision: PB.
Visualization: NCC PB.
Writing – original draft: NCC.
Writing – review & editing: NCC PB.

References
1. Colston MJ. The Microbiology of Mycobacterium leprae, progress in the last 30 years. Trans of the Royal Soc of Trop Med Hyg 1993; 87: 504–507. doi: 10.1016/0035-9203(93)90064-W PMID: 8266397
2. de las Aguas T. Historia de la Terapéutica de la Lepra. Leprología. 2001; 4: 117–124.
3. Gelber RH; Grosset J. The chemotherapy of leprosy: An interpretive history. Lepr Rev 2010; 83: 221–240.
4. World Health Organization Study Group. Chemotherapy of leprosy for control programmes. WHO Technical Report Series Geneva. 1982; 675: 1–36.
5. Sekar B, Elangeswaran N, Jayarama E, Rajendran M, Kumar SS, Vijayaraghavan R, Anandan D, Arunagiri K. Drug susceptibility of Mycobacterium leprae: a retrospective analysis of mouse footpad inoculation results from 1983 to 1997. Lepr Rev. 2002; 73: 239–244. PMID: 1244988
6. Roche PW, Neupane KD, Failbus SS, Butlin CR. Dapsone drug resistance in the MDT era. Int J Lepr Other Mycobact Dis. 2000; 68: 323–325. PMID: 11221097
7. Cardona-Castro N. Leprosy in Colombia: Post Elimination Stage? Lepr Rev. 2013; 84: 1–10. PMID: 24428118
8. Protocolos de vigilancia en salud pública. Lepra. Secretaría Distrital de Salud de Bogotá. Dirección de salud pública.http://www.saludcapital.gov.co/sitios/VigilanciaSaludPublica/Protocolos%20de%20Vigilancia%20en%20Salud%20Publica/Lepra.pdf Accessed April 25, 2016.
9. Plan Estratégico de Colombia sobre Lepra 2010–2015. Plan estratégico de Colombia para aliviar la carga de la enfermedad y sostener las actividades de control de lepra 2010–2015 http://www.paho.org/COL/index.php?option=com_content&view=article&id=504:plan-estrategico-de-colombia-sobre-lepra-2010-2015&catid=753&Itemid=470 Accessed April 25, 2016.
10. Hernández E, Cardona-Castro N, Rodríguez G, Villegas S, Beltrán C, Kimura M, Vissa VD, Gómez Y. Estudio de resistencia a la rifampicina y la dapsona en tres pacientes con recurrencia de lepra. Rev Panam Salud Publica. 2008; 23: 73–77. doi: 10.1590/S1020-49892008000200001 PMID: 18371276
11. Guerrero MI, Colorado CL, Torres JF, León CI. Is drug-resistant Mycobacterium leprae a real cause for concern? First approach to molecular monitoring of multibacillary Colombian patients with and without previous leprosy treatment. Biomédica. 2014; 34: 137–47. doi: 10.1590/S0120-41572014005000016 PMID: 24968045
12. dela Cruz E, Cellona RV, Balagon MV, Villahermosa LG, Fajardo TT Jr, Abalos RM, Tan EV, Walsh GP. Primary dapsone resistance in Cebu, the Philippines: cause for concern. Int J Lepr Other Mycobact Dis. 1996; 64: 253–256. PMID: 8982298
13. Honoré N, Cole ST. Molecular basis of rifampicin resistance in Mycobacterium leprae. Antimicrob Agents Chemother. 1993; 37: 414–418. doi: 10.1128/aaac.37.3.414 PMID: 8460911
14. Gillis TP, Williams DL. Dapsone resistance in Mycobacterium leprae. Lepr Rev. 2000; 71: 91–95. doi: 10.5935/0305-7518.20000078
15. Cambau E, Carthagena L, Chauffour A, Ji B, Jarlier V. Dihydropteroate synthase mutations in the folP1 gene predict dapsone resistance in relapsed cases of leprosy. Clin Infect Dis. 2006; 42: 238–241. doi: 10.1086/498506 PMID: 16355335
16. Yano T, Kassovska-Bratinova S, Teh JS, Winkler J, Sullivan K, Isaacs A, Schechter NM, Rubin H. Reduction of clofazimine by mycobacterial type 2 NADH:quinone oxidoreductase: a pathway for the generation of bactericidal levels of reactive oxygen species. J Biol Chem. 2011; 286: 10276–87. doi: 10.1074/jbc.M110.200501 PMID: 21193400
17. Nakata N, Kai M, Makino M. Mutation analysis of the Mycobacterium leprae folP1 gene and dapsone resistance. Antimicrob Agents Chemother. 2011; 55: 762–766. doi: 10.1128/AAC.01212-10 PMID: 21115799
18. World Health Organization. Drug resistance in leprosy: reports from selected endemic countries. Weekly epidemiological record. 2009; 84: 264–267. http://www.who.int/wer/2009/wer8433.pdf?ua=1 Accessed May 3, 2016. PMID: 19557943
19. World Health Organization Regional Office for South-East Asia. Guidelines for global surveillance of drug resistance in leprosy. SEA-GLP 2:1–32. 2009. http://www.searo.who.int/entity/global_leprosy_programme/publications/guide_surv_drug_res_2009.pdf Accessed Accessed May 3, 2016.

20. Cellona RV, Balagon MF, de la Cruz EC, Burgos JA, Abalos RM, Walsh GP, Topolski R, Gelber RH, Walsh DS. Long-term efficacy of 2 year WHO multiple drug therapy (MDT) in multibacillary MB leprosy patients. Int J Lepr Other Mycobact Dis 2003; 71: 308–19. doi: 10.1489/1544-581X(2003)071<0308:LEOYW>2.0.CO;2 PMID: 14763888

21. QIAGEN®. DNeasy® Blood & Tissue Handbook. 2006; Available at: http://www.qiagen.com/products/genomicdnastabilizationpurification/dneasytissuesystem/dneasybloodtissuekit.aspx#Tabs=t2. Accessed November 1, 2015.

22. QIAGEN®. Qiagen Multiplex PCR Handbook. 2008; Available at: http://www.qiagen.com/products/pcr/multiplexsystem/multiplexpcr.aspx#Tabs=t2. Accessed November 1, 2015.

23. Technelysium Pty Ltd. Chromas lite. 2005; Available at: http://www.technelysium.com.au/chromas_lite.html. Accessed November 1, 2015.

24. World Health Organization. Transmission of leprosy. 2010; Available at: http://www.who.int/lep/transmission/en/index.html. Accessed November 1, 2015.

25. Matsuoka M, Budiawan T, Aye KS, Kyaw K, Tan EV, Cruz ED, Gelber R, Saunders P, Balagon V, Pannikar V. The frequency of drug resistance mutations in Mycobacterium leprae isolates in untreated and relapsed leprosy patients from Myanmar, Indonesia and the Philippines. Lepr Rev. 2007; 78: 343–352. PMID: 18309708

26. Eun-Young Y, Tae-Jin K, Se-Kon K, Seong-Beom L, Gue-Tae C. Mutations in genes related to drug resistance in Mycobacterium leprae isolates from leprosy patients in Korea. J Infect Dis. 2005; 50: 6–11. doi: 10.1016/j.jinf.2004.03.012 PMID: 15603834

27. Lopez-Roa RI, Fafurtis-Morris M, Matsuoka M. A drug resistant leprosy case detected by DNA sequence analysis from a relapsed Mexican leprosy patient. Rev Latinoam Microbiol. 2006; 48: 256–259. PMID: 18293659

28. Cambau E, Perani E, Guillemin I, Jamet P, Ji B. Multidrug-resistance to dapsone, rifampicin, and ofloxacin in Mycobacterium leprae. The Lancet. 1997; 349: 103–104. doi: 10.1016/S0140-6736(05)60888-4 PMID: 8996430

29. Honoré N, Roche PW, Grosset JH, Cole ST. A method for rapid detection of rifampicin-resistant isolates of Mycobacterium leprae. Lepr Rev. 2001; 72: 441–448. doi: 10.1016/S0140-6736(05)60888-4 PMID: 11826480

30. Cambau E, Bonnafous P, Perani E, Sougakoff W, Ji B, Jarlier V. Molecular Detection of Rifampicin and Ofloxacin Resistance for Patients Who Experience Relapse of Multibacillary Leprosy. Clin Infect Dis. 2002; 34: 39–45. doi: 10.1086/324623 PMID: 11731943

31. Dauendorffer JN, Guillemin I, Aubry A, Truffot-Pernot C, Sougakoff W, Jarlier V, Cambau E. Identification of Mycobacterial species by PCR sequencing of quinolone resistance-determining regions of DNA gyrA genes. J Clin Microbiol. 2003; 41: 1311–1315. doi: 10.1128/JCM.41.3.1311-1315.2003 PMID: 12624075

32. Saunders P. Drug-resistance M leprae. Clinics in Dermatology. 2016; 34: 79–81. doi: 10.1016/j.clindermatol.2015.10.019 PMID: 26773627

33. Rodríguez G, Pinto R, Laverde C, Sarmiento M, Riveros A, Valderrama J, Ordóñez N. Recidivas post-ratamiento de la lepra multibacilar. Biomedica. 2004; 24: 133–139. doi: 10.7705/biomedica.v24i2.1259

34. Singh P, Benjak A, Carat S, Kai M, Busso P, Avanzi C, Paniz-Mondolfi A, Peter C, Harshman K, Rougemont J, Matsuoka M, Cole ST. Genome-wide re-sequencing of multidrug-resistant Mycobacterium leprae Airaku-3. Clin Microbiol Infect. 2014; 20(10):619–22. doi: 10.1111/1469-0691.12609 PMID: 24612452

35. Jong-Pill K, Yeon-Sil K. Mutation of rpoB gene in M. leprae isolates from Korea. Korean Leprosy Bulletin. 2007; 40: 3–13.

36. Nakata N, Kai M, Makino M. Mutation Analysis of Mycobacterial rpoB Genes and Rifampicin Resistance Using Recombinant Mycobacterium smegmatis. Antimicrobial Agents and Chemotherapy. 2012; 56: 2008–2013 doi: 10.1128/AAC.05831-11 PMID: 22252831

37. Matrat S, Cambau E, Jarlier V, Aubry A. Are all the DNA mutations found in Mycobacterium leprae clinical strains involved in resistance to fluoroquinolones. Antimicrob Agents Chemother. 2008; 52: 745–747. doi: 10.1128/AAC.01095-07 PMID: 18070975

38. Se-Kon K, Seong-Beom L, Tae-Jin K, Gue-Tae C. Detection of gene mutations related with drug resistance in Mycobacterium leprae from leprosy patients using Touch-Down PCR. FEMS Immunol Med Microbiol. 2003; 36: 27–32. doi: 10.1016/s0928-8244(03)00045-6 PMID: 12727362
39. Monot M, Honoré N, Garnier T, Zidane N, Sherafi D, Paniz-Mondolfi A, Matsuoka M, Taylor GM, Donoghue HD, Bouwman A, Mays S, Watson C, Lockwood D, Khamisiypour A, Dowlati Y, Jianping S, Rea TH, Vera-Cabrera L, Stefani MM, Banu S, Macdonald M, Sapkota BR, Spencer JS, Thomas J, Harshman K, Singh P, Busso P, Gattiker A, Rougemont J, Brennan PJ, Cole ST. Comparative genomic and phylogeographic analysis of Mycobacterium leprae. Nature Genetics. 2009; 41, 1282–1289. doi: 10.1038/ng.477 PMID: 19881526

40. Takiff HE, Salazar L, Guerrero C, Philipp W, Huang WM, Kreiswirth B, Cole ST, Jacobs WR Jr, Telenti A. Cloning and nucleotide sequence of Mycobacterium tuberculosis gyrA and gyrB genes and detection of quinolone resistance mutations. Antimicrob Agents Chemother. 1994; 38:773–780. doi: 10.1128/aac.38.4.773 PMID: 8031045

41. Mani V, Wang S, Inci F, De Libero G, Singhal A, Demirci U. Emerging technologies for monitoring drug-resistant tuberculosis at the point-of-care. Adv Drug Deliv Rev. 2014; 78C: 105–117. doi: 10.1016/j.addr.2014.05.015 PMID: 24882226

42. Cambau E, Chauffour-Nevejans A, Tejmar-Kolar L, Matsuoka M, Jarlier V. Detection of antibiotic resistance in leprosy using GenoType Leprae DR, a novel ready-to-use molecular test. PLoS Negl Trop Dis. 2012; 6 (7):e1739. doi: 10.1371/journal.pntd.0001739 Epub 2012 Jul 31. PMID: 22860144