Lepr Rev (2025) **96,** e2025110 doi:10.47276/lr.96.4.2025110

Molecular detection of mutations in the *gyrA* gene associated with fluoroquinolone resistance in *Mycobacterium leprae* strains from leprosy patients in Adzopé, Côte d'Ivoire

Dehe Bahou Roger[a,b], Amon Aby Christiane[b], Tiémoko Gogbé Jean-Luc[c], Ban Ouéméla Venance Allais[c], Kouakou Henry[d], Kakou-N'Gazoa Solange[b] & Coulibaly N'Golo David[b]

[a]*UFR Marine Sciences, Laboratory of Oceanographic and Marine Resources, San Pedro University, San Pedro, Côte d'Ivoire*
ORCID: https://orcid.org/0009-0006-0448-5609
[b]*Molecular Biology Platform, Pasteur Institute of Côte d'Ivoire, BP 490 Abidjan, Abidjan, Côte d'Ivoire*
[c]*UFR Agriculture, Fishery Ressources and Agro-Industries, San Pedro University, San Pedro, Côte d'Ivoire*
[d]*Follereau Raoul Institute of Côte d'Ivoire, Adzope, Côte d'Ivoire*

Submitted 17 September 2025; Accepted 21 October 2025

Summary
Objectives Recommended control measures for leprosy treatment with multidrug therapy (MDT) aim to limit the spread of drug-resistant *Mycobacterium leprae* strains. However, fluoroquinolone resistance continues to be reported in several endemic countries. In Côte d'Ivoire, no study has yet evaluated *M. leprae* resistance to fluoroquinolones used in MDT. This study provides the first assessment of fluoroquinolone resistance in *M. leprae* isolates collected in Adzopé, Côte d'Ivoire.
Methods For each clinically confirmed case of multibacillary leprosy (new cases and relapses), slit-skin smear samples were collected and analyzed by microscopic examination after Ziehl-Neelsen staining, as well as by PCR targeting the RLEP repeat sequence. A total of 69 isolates were tested for amplification of the *gyrA* gene, known to be involved in fluoroquinolone resistance. Mutations were identified by sequencing of the amplified regions using the ABI 3500 genetic analyzer.
Results Among the 69 isolates analyzed, 9 (18.75%) harboured mutations conferring resistance to fluoroquinolones. The mutation detected was A91V (9.09%), which is known to be associated with fluoroquinolone resistance.
Conclusions This study provides evidence of fluoroquinolone-resistant *M. leprae* strains in Côte d'Ivoire. These findings highlight the need for regular molecular

Correspondence to: Dehe Bahou Roger (e-mail: b_dehe@yahoo.com; dehe.bahou@usp.edu.ci)

surveillance to monitor the evolution of drug resistance and support the development of control strategies to limit the spread of resistant strains in endemic areas.

Keywords: Leprosy, mycobacterium leprae, fluoroquinolone resistance, resistance gene, mutations

Introduction

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*, an obligate intracellular pathogen capable of infecting skin cells and peripheral nerves.[1] Leprosy was diagnosed in 172,717 new cases worldwide in 2024 and remains responsible for irreversible nerve damage, often linked to late diagnosis.[2]

Despite efforts deployed by the World Health Organization (WHO) to eliminate the disease, the transmission chain has not been completely interrupted, even after the introduction of multidrug therapy (MDT). Moreover, no effective vaccine is currently available, as *M. leprae* cannot be cultured on artificial media, limiting possibilities for advanced research on this pathogen. Consequently, drug therapy remains the main strategy in the fight against leprosy.

Since 1982, the WHO has recommended MDT based on the combination of rifampicin, dapsone, and clofazimine.[3] This treatment has significantly reduced the prevalence of treated cases but has had limited impact on the incidence of new cases.[4] Furthermore, increasing reports of relapse cases and the emergence of mutant *M. leprae* strains have been documented.[5]

Recent studies in several African countries have highlighted the emergence of drug-resistant and multidrug-resistant strains of *M. leprae*. What is the situation in Côte d'Ivoire? Several factors may promote the development of drug resistance in the country, including the continued use of inappropriate monotherapy in the private sector, misconceptions about the disease, and a lack of awareness among the population. These risk factors are particularly present in Côte d'Ivoire, where MDT has been deployed for several years, raising concerns about the emergence of drug-resistant *M. leprae* strains, as reported in other countries.

It is worth noting that few studies have been conducted in Côte d'Ivoire to assess *M. leprae* susceptibility to fluoroquinolones, a class of antibiotics used as an alternative treatment or in cases of resistance.[3]

Resistance to the main anti-leprosy drugs, rifampicin and dapsone, has been known since the 1960s.[6] Traditionally, drug susceptibility testing was performed using the mouse footpad method, a laborious and time-consuming technique.[7] However, the advent of molecular biology tools has enabled rapid detection of mutations associated with drug resistance, particularly through direct sequencing of drug resistance-determining regions (DRDR). Although fluoroquinolones are not part of standard MDT, they are important second-line drugs. Resistance may develop when the drugs are used to treat other infections in someone who is unknowingly incubating leprosy. One recent global study found rifampicin resistance in 2% and fluoroquinolone resistance in 1% of new, untreated cases.[5]

Since 2011, several countries have incorporated these molecular tools into their surveillance systems for *M. leprae* drug resistance.[8] The molecular targets of the different anti-leprosy drugs are now well defined. In particular, resistance to fluoroquinolones is associated with mutations in the *gyrA* gene, encoding the A subunit of DNA gyrase.[9]

Antimicrobial resistance (AMR) surveillance has become a key component of global leprosy control programs. The availability of local resistance data is essential for monitoring resistance trends in both new and relapsed cases.[8] In 2008, the WHO established guidelines for

global surveillance of *M. leprae* drug resistance, recommending the use of PCR-sequencing methods.

In light of the observed relapse cases in Côte d'Ivoire potentially due to poor treatment adherence or circulation of resistant strains, we evaluated fluoroquinolone susceptibility in 69 patients attending a leprosy care center in Adzopé (Raoul Follereau Center). This study reports our findings on *gyrA* gene mutations detected in *M. leprae* strains isolated from clinical samples collected in this region.

Our study was limited to fluoroquinolone resistance due to financial constraints, unavailability of some reagents (e.g., primers for dapsone and rifampicin resistance), and because this work represents the pilot phase of a broader antimicrobial resistance surveillance program in Côte d'Ivoire.

Material and methods

STUDY POPULATION AND SAMPLING

The various samples used in this study were collected from patients attending the Raoul Follereau Institute of Côte d'Ivoire (IRFCI) in Adzopé. The study included multibacillary leprosy patients, whether or not they were receiving anti-leprosy chemotherapy at the time of sampling. The inclusion criteria were multibacillary leprosy with a positive bacteriological index, in accordance with the WHO protocol.[10] In total, 69 dermal fluid samples were collected from both relapse cases and newly diagnosed leprosy patients.

BIOLOGICAL SAMPLES

The biological samples analyzed in this study consisted of slit-skin smears. The slit-skin smear was collected from each earlobe of the patients and preserved in 2 ml microtubes containing 500 µl of phosphate-buffered saline (PBS).

DNA EXTRACTION FROM *M. LEPRAE*

Bacterial DNA was extracted from the samples using a modified guanidine thiocyanate protocol as previously described.[11] Briefly, 300 µl of each sample were pretreated by boiling at 95°C for thirty minutes. The DNA was then extracted using a lysis buffer containing 5M guanidine thiocyanate, 50 mM Tris (pH = 8.0), 10 mM EDTA, 5% 2-mercaptoethanol, and 2% Triton X-100. After centrifugation, the supernatant was transferred to sterile 1.5 ml Eppendorf tubes, and the DNA was precipitated using isopropanol and sodium acetate (3M). The DNA was then washed with absolute ethanol (96%) and finally resuspended in 150 µl of Tris-EDTA (TE) buffer. The concentration and purity of the samples were evaluated using the Qubit 3 equipment (Invitrogen).

CASE CONFIRMATION

Leprosy cases were confirmed by conventional PCR targeting the repetitive *Mycobacterium leprae* element RLEP as previously described.[12] The PCR was performed in a final volume of 25 µl containing 5 µl of DNA template, 3 mM MgCl , 0.4 µM of each primer, 0.6 mM dNTPs, 2X buffer, and 0.04 U/µl of polymerase. PCR conditions were carried out in a GeneAmp 9700 PCR System (Applied Biosystems) with the following program: an initial denaturation at 94 °C for 5 min, followed by 35 cycles consisting of denaturation at 94°C for 30 s, annealing at 57°C for 30 s, extension at 72°C for 60 s, and a final extension at 72 °C for 10 min. The PCR products (545 bp) were visualized using a GelDoc EZ imager (BioRad) after electrophoresis on a 2%

agarose gel containing SybrGreen. Molecular drug sensitivity testing was only conducted on RLEP-PCR-positive patients with a bacilloscopic index greater than or equal to +1.

AMPLIFICATION AND SEQUENCING ANALYSIS OF A PORTION OF *GYRA*

PCR was performed using GoTaq polymerase (PROMEGA, Madison, WI, USA) in a 25 µl reaction volume containing 5 µl of genomic DNA and 10 µM concentrations of primers designed according to the *M. leprae gyrA* gene sequence (Z70722). The primers for the *gyrA* gene were *gyrA*-1 (5′-ATGACTGATATCACGCTGCCA-3′) and *gyrA*-2 (5′-ATAACGCATCGCTGCCGGTGG-3′), designed to amplify a 398 bp fragment of the *gyrA* gene as previously described for detection of ofloxacin resistance.[5] The target region of the *gyrA* gene was amplified using a GeneAmp® PCR System 9700 (Applied Biosystems, Singapore) with a program of 30 s at 95°C, 2 min at 50°C, and 3 min at 72°C for 40 cycles. A no-DNA control (reagent control) was included as a negative control. The PCR products containing the amplified fragments of the target regions were subjected to electrophoresis in a 3% agarose gel (Sigma) using Tris-Borate-EDTA (TBE) buffer, with a constant voltage of 110 V for one hour. PCR products were recovered from the agarose gel, purified using the GFX PCR DNA and Gel Band Purification Kit according to the manufacturer's protocol, and sequenced using the same primers as those used for PCR amplification with the Big Dye Terminator V3.1 reaction kit (Cycle Sequencing Kit reference 4336917). Sequences were generated on the ABI 3500 Genetic Analyzer (24 capillaries, Applied Biosystems) and compared to the *M. leprae* NC002677 and *gyrA* (Z70722) sequences available at GenBank (http://www.ncbi.nlm.nih.gov). For mutation zone analysis, sequences were introduced into the Mega 7 software. Additionally, 48 out of 69 samples showed adequate results for sequencing.

EFFECT OF MUTATIONS ON OFLOXACIN

The impact of each mutation was analyzed using the antimicrobial resistance profiles for Hansen's disease (HARP).[13]

Results and discussion

Ziehl-Neelsen staining was performed on all previously processed samples. The bacilloscopic examination of all patients ranged from 1+ to 6+. All these samples contained acid-fast bacilli. For case confirmation, the detection of *M. leprae* by PCR targeting the RLEP region resulted in 69 positive samples, representing 100% of the samples. Figure 1 shows an agarose gel displaying the result of amplicon migration following the PCR reaction on a 2% gel. PCR identification gave 52 positive samples, which corresponds to 75.66% of the total samples. Only 48 DNA extracts from the 52 PCR-positive samples targeting the *gyrA* gene were used for sequencing. This selection was based on the quality of the fragments present in the agarose gel. The most DNA-rich extracts were used. Figure 2 shows the result of amplicon migration on a 3% agarose gel.

Thus, 48 informative sequences were obtained for *gyrA* after sequencing. Drug-associated mutations were detected in 9 out of the 48 isolates for the *gyrA* gene (18.75%). Regarding the nature of the mutations, the 9 *gyrA* changes exhibited a GCA to GTA transition (A to V) at codon 91 (GenBank: PX399127.1) (Table 1). The nine cases of resistance were predominantly relapse cases. The nucleotide composition changes resulting from the mutations lead to the changes in the amino acid composition of the protein encoded by the *gyrA* gene of *M. leprae* (Table 2).

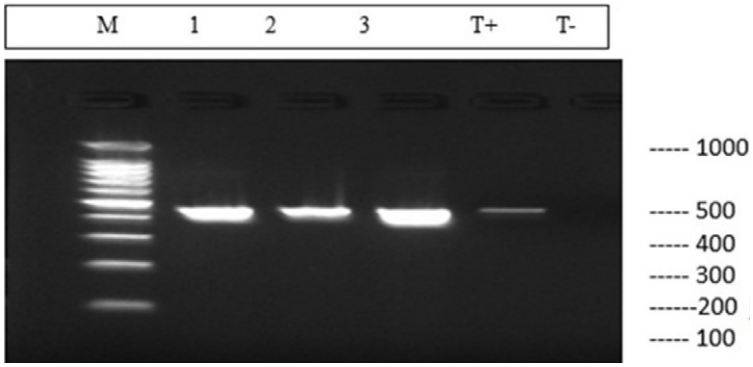

Figure 1. PCR amplification of the RLEP gene (545 bp). Lane M: 100 bp DNA molecular weight marker; lanes 1–3: positive clinical samples; lane T−: negative control containing DNA-free water; lane T+: positive control containing *M. leprae* DNA.

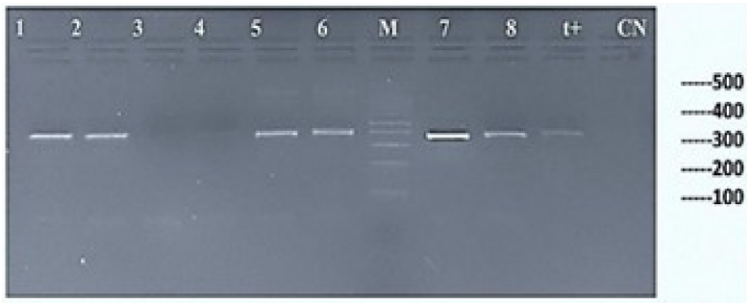

Figure 2. PCR amplification of the *gyrA* gene on 3% agarose gel. Lane M: 100 bp DNA molecular weight marker; lane T+: positive control containing *M. leprae* DNA; lanes 1–8: clinical samples; lane CN: negative control containing DNA-free water.

Table 1. Results of *gyrA* gene amplification and sequencing

Gene	PCR positivity (%) (number of samples = 69)	Number of samples with mutations (%)
gyrA	52 (75.66%)	9/48 (18.75%)

Table 2. Mutations in *gyrA* gene by category

Category	Number of PCR-positive cases (out of 48)	Number of mutations (mutation rate)	Type of mutation(s) detected
New case	16	-	-
Relapse	16	9 (27.08%)	9 (91 : GCA-GTA)
Old cases	16	-	-

The *gyrA* gene encodes the formation of DNA gyrase, which plays a role in the topological regulation of DNA. The presence of certain mutations in this gene can lead to resistance

to ofloxacin. The A91V-resistant mutants were used as comparators in the analysis of the mutation effect on ofloxacin. The A91V mutation was located near the DNA gyrase binding cavity, at a distance of 3.5 Å.[14] These mutations cause destabilization of the protein and have a significant overall impact.

Mutations in the *gyrA* gene region that determine resistance to quinolones have also been reported for certain mycobacteria.[15] Ofloxacin is a second-line anti-leprosy drug.[16] Single-dose ofloxacin has been used in several countries, such as India, Bangladesh, and Brazil.[16] The limitation of surveillance coverage in high-burden leprosy countries, such as India and Brazil, has become a concern due to the potential risk of resistance.[5] A single case of quinolone resistance in leprosy was reported in Mali.[17] A mutation (Ala → Val at position 91) was detected in five *M. leprae* isolates, and another mutation (Gly → Cys at position 89) was found in *M. tuberculosis* resistant to quinolones. The mutation observed in this study has been reported in other countries, including changes in the codon causing a mutation at codon 91 of *gyrA* (n = 9), leading to an Ala to Val change. This base substitution had previously been described in several reports, including those by Jamal.[15,18–20] In addition to the non-synonymous SNP (single nucleotide polymorphism) in *gyrA*, the distribution of alleles in relapse cases of a recently observed synonymous SNP at position 297 of *gyrA* was noted,[21,22] showing that 74% of cases carried *M. leprae* of the *gyrA* SNP type C at position 297. Published data by Singh[22] revealed a correlation between the synonymous SNP *gyrA* 297T and SNP type 3, and the SNP *gyrA* C with types 1 or 4, as defined by Chauffour.[20] Previous data showed a higher frequency of SNP type 3 in southeastern Brazil[23] and Latin America.[22]

The mutation in the *gyrA* gene, observed in 9 *M. leprae* isolates and associated with ofloxacin resistance, is the same as that observed in *in vitro* mutants of *M. smegmatis*.[24] Substitutions at position 91 in the A subunit of DNA gyrase are commonly associated with quinolone resistance due to a decreased affinity of DNA gyrase for quinolones.[25] This research plays an important role in ensuring the efficacy of using ofloxacin if it becomes an alternative treatment for leprosy.

Conclusion

A variant in the *gyrA* gene of *M. leprae* was found in this study. This variant is A91V. However, an *in vivo* test is needed to validate these results. The presence of mutations in the *gyrA* gene of *M. leprae* discovered in this study could provide useful information for leprosy treatment in Côte d'Ivoire if ofloxacin is used as an alternative treatment. Furthermore, the existence of mutations also provides information to enhance vigilance and improve patient adherence during the treatment process to prevent the emergence of drug-resistant *M. leprae* strains.

Acknowledgements

The authors would like to thank the direction of the Pasteur Institute of Côte d'Ivoire for financial assistance and the Raoul Follereau Institute Côte d'Ivoire who provided sample collection and microbiology analysis.

Ethical considerations

This study was approved by the National Ethics Committee for Research of Côte d'Ivoire under the approval number N/Ref: No. 140/MSHP/CNER-km. All participants agreed to the research protocol and signed the informed consent form after reading the study information sheet.

Conflict of interest

The authors declare that they do not have any conflict of interests.

Funding

This study was entirely funded by the Pasteur Institute of Côte d'Ivoire.

Authors' contributions

Conceptualization, CND and DBR; methodology, DBR, AAC, TGJL and KH; software, DBR, TGJL and BOV; validation, CND; formal analysis, CND, DBR, TGJL; KH, data curation, NKS and DBR; writing-original draft preparation, DBR, CND; writing-review and editing, DBR, DM, TGJL, KH; supervision, CND; project administration, CND; All authors have read andagreed to the published version of the manuscript.

Supplementary data

Supplemental information for this article can be found online at https://doi.org/10.47276/lr.96.4.2025110.

References

[1] Azevedo MC, Ramuno NM, Fachin LR, Tassa M, Rosa PS, Belone AF, Diorio SM, Soares CT, Garlet GP, Trombone AP. qPCR detection of *Mycobacterium leprae* in biopsies and slit skin smear of different leprosy clinical forms. *Braz J Infect Dis*, 2017; **21**(1): 71–78.

[2] World Health Organization. Global leprosy (Hansen disease) update, 2023: rising to the challenges. *Wkly Epidemiol Rec*, 2024; **99**(37): 501–521.

[3] David CN, Roger DB, Solange K-N, Henry K, Christiane AA, Aboubacar S, Philippe BAD, Vagamon B, Mireille D. Drugs susceptibility testing in leprosy patients from Côte d'Ivoire reveals multidrugs resistance combination cases to dapsone, rifampicin and ofloxacin. *Am J Microbiol Res*, 2020; **8**: 160–163. doi:10.12691/ajmr-8-4-6.

[4] World Health Organization. Global leprosy update, 2019: accelerating reduction of disease burden. *Wkly Epidemiol Rec*, 2020; **92**(34): 501–520.

[5] Cambau E, Saunderson P, Matsuoka M, Cole ST, Kai M, Suffys P, Rosa PS, Williams D, Gupta UD, Lavania M, Cardona-Castro N, Miyamoto Y, Hagge D, Srikantam A, Hongseng W, Indropo A, Vissa V, Johnson RC, Cauchoix B, Pannikar VK, Cooreman EAWD, Pemmaraju V, Gillini L. Resistance to antimicrobials in leprosy: results of the first open prospective survey conducted by the WHO surveillance network for the period 2009-2015. *Clin Microbiol Infect*, 2018; **24**: 1305–1310.

[6] Maeda S, Matsuoka M, Nakata N, Kai M, Maeda Y, Hashimoto K, Kimura H, Kobayashi K, Kashiwabara Y. Multidrug resistant *Mycobacterium leprae* from patients with leprosy. *Antimicrob Agents Chemother*, 2001; **45**(12): 3635–3639.

[7] Levy L, Ji B. The mouse foot-pad technique for cultivation of *Mycobacterium leprae*. *Lepr Rev*, 2006; **77**(1): 5–24. Erratum in: *Lepr Rev.* 2006; 77(2): 170. PMID: 16715686.

[8] World Health Organization. *A Guide for Antimicrobial Resistance Surveillance in Leprosy: Update 2017*. Geneva: WHO, 2017. 56 p.

[9] Guerrero MI, Colorado CL, Torres JF, Leon 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. *Biomedica*, 2014; **34**: 137–147.

[10] World Health Organization. Department of Control of Neglected Tropical Diseases. Global leprosy situation. *Weekly Epidemiological Record*, 2009; **33**(84): 333–340.

[11] Yoshikawa H, Dogruman-Ai F, Turk S, Kustimur S, Balaban N. Evaluation of DNA extraction kits for molecular diagnosis of human Blastocystis subtypes from fecal samples. *Parasitol Res*, 2011; **109**(4): 1045–1050.

[12] Amon AC. Molecular diagnosis of leprosy in Côte d'Ivoire: detection of *Mycobacterium leprae* from various types of samples in leprosy patients [Master's thesis]. Abidjan: Felix Houphouet Boigny Université, 2016, 50 p.

[13] Moraes MO. Editorial commentary: drug-resistance in leprosy: moving toward understanding the scope of the problem and how to tackle it. *Clin Infect Dis*, 2016; **63**(11): 1485–1486.

[14] Cabic E, Cabic AG, Esposo SM, Dizon F, Quinones GJ, Guia A. Histopathological detection of *Mycobacterium tuberculosis* and *Mycobacterium leprae* using a modified acid-fast technique. *Philipp J Pathol*, 2018; **3**(1): 30–33.

[15] Jamal MA, Maeda S, Nakata N, Kai M, Fukuchi K, Kashiwabara Y. Molecular basis of clarithromycin resistance in the *Mycobacterium avium*-intracellulare complex. *Tuberc Lung Dis*, 2010; **80**: 1–4.

[16] André E, Goeminne L, Cabibbe A, Beckert P, Kabamba MB, Mathys V. Consensus numbering system for mutations in rpoB gene associated with rifampicin resistance in pathogenic mycobacteria. *Clin Microbiol Infect*, 2017; **23**(3): 167–172.

[17] Traoré B, Cissé L, Fofana Y, Coulibaly A, Somboro D, Karabinta Y. Squamous cell carcinoma on leprous ulcers: study of 19 cases. *Bull ALLF*, 2018; **33**: 80–88.

[18] Williams D, Hagino T, Sharma R, Scollard D. Primary multidrug-resistant leprosy, United States. *Emerg Infect Dis*, 2013; **19**: 179–181.

[19] Lavania M, Hena A, Reja H. Mutation at codon 442 in the rpoB gene of *Mycobacterium leprae* does not confer resistance to rifampicin. *Lepr Rev*, 2016; **87**(1): 93–100.

[20] Chauffour A, Lecorche E, Reibel F, Mougari F, Raskine L, Aubry A, Jarlier V, Cambau E. Prospective study on antimicrobial resistance in leprosy cases diagnosed in France from 2001 to 2015. *Clin Microbiol Infect*, 2018; **24**(11): 1213.

[21] Da Silva Rocha A, Diniz LM, Cunha M, Salgado C. Genotyping of *Mycobacterium leprae* in leprosy patients in Brazil suggests the occurrence of reinfection or changes in the bacterial population during relapse. *J Med Microbiol*, 2011; **60**: 1441–1446.

[22] Singh P, Rivas C, Cole ST. Molecular drug susceptibility testing and genotyping of *Mycobacterium leprae* strains from South America. *Antimicrob Agents Chemother*, 2011; **55**: 2971–2973.

[23] Veziris N, Chauffour A, Escolano S, Henquet S, Matsuoka M, Jarlier V, Aubry A. *Mycobacterium leprae* resistance to quinolones: a matter of relativity? *PLoS Negl Trop Dis*, 2013; **7**: e259-81.

[24] Smith CS, Aerts A, Saunderson P, Kawuma J, Kita E, Virmond M. Multidrug therapy for leprosy: a decisive factor in elimination. *Lancet Infect Dis*, 2017; **17**(9): 293–297.

[25] Mejía MD, Santos MP, Da Silva GA. Identification of primary drug resistance to rifampin in *Mycobacterium leprae* strains from leprosy patients in Amazonas State. *Brazil J Clin Microbiol*, 2014; **52**: 4359–4360.