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## **A systematic review of *Mycobacterium leprae* DNA gyrase mutations and their impact on fluoroquinolone resistance**

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1 **Intended category:** systematic review

2

3 A systematic review of *Mycobacterium leprae* DNA gyrase mutations and their impact on  
4 fluoroquinolone resistance.

5

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33

34 **ABSTRACT**

35 **Background**

36 The fact that *M. leprae* does not grow *in vitro* remains a challenge in the survey of its  
37 antimicrobial resistance (AMR). Mainly molecular methods are used to diagnose AMR in *M.*  
38 *leprae* to provide reliable data concerning mutations and their impact. Fluoroquinolones (FQ)  
39 are efficient for the treatment of leprosy and the main second-line drugs in case of multidrug  
40 resistance.

41 **Objectives**

42 This study aimed at performing a systematic review (i) to characterize all DNA gyrase gene  
43 mutations described in clinical isolates of *M. leprae* and (ii) to distinguish between those  
44 associated with FQ resistance or susceptibility, and (iii) to delineate a consensus numbering  
45 system for *M. leprae* GyrA and GyrB.

46 **Data sources**

47 Data source was PubMed.

48 **Study eligibility criteria**

49 Publications reporting genotypic susceptibility-testing methods and gyrase gene mutations in  
50 *M. leprae* clinical strains.

51 **Results**

52 In 25 studies meeting our inclusion criteria, 2884 *M. leprae* isolates were analyzed (2236 for  
53 *gyrA* only (77%) and 755 for both *gyrA* and *gyrB* (26%)):3.8% of isolates had *gyrA* mutations  
54 (n = 110), mostly at position 91 (n = 75, 68%) and 0.8% *gyrB* mutations (n = 6). Since we found  
55 discrepancies regarding the location of substitutions associated with FQ-resistance, we  
56 established a consensus numbering system to properly number the mutations. We also designed  
57 a 3D model of the *M. leprae* DNA gyrase to predict the impact of mutations whose role in FQ-  
58 susceptibility has not been demonstrated previously.

59 **Conclusion**

60 Mutations in DNA gyrase are observed in 4% of the *M. leprae* clinical isolates. To solve  
61 discrepancies among publications and to distinguish between mutations associated with FQ  
62 resistance or susceptibility, the consensus numbering system we proposed as well as the 3D  
63 model of the *M. leprae* gyrase for the evaluation of the impact of unknown mutations in FQ  
64 resistance, will provide help for resistance surveillance.

65

66 Keywords: *Mycobacterium leprae*, resistance, fluoroquinolones, GyrA, GyrB, mutations,  
67 substitutions

68

## 69 INTRODUCTION

70 *Mycobacterium leprae*, the etiological agent of leprosy, was responsible for 193 840 new cases  
71 in 2019. [1] Additionally, in 2019, 3897 cases of leprosy relapses were reported by 55 countries,  
72 representing 2% of the total case notification. [1] Relapses can be due to non-adherence to the  
73 recommended multidrug therapy (MDT) or to antimicrobial resistance (AMR). [2–5]  
74 Monitoring AMR remains challenging because *M. leprae* does not grow *in vitro*. Two methods  
75 can be used for AMR monitoring: an *in vivo* phenotypic method using Shepard’s mouse footpad  
76 model [6] which requires a high level of expertise and is expensive and time-consuming (ca. 12  
77 months); or genotypic methods, such as PCR sequencing, a line probe assay (i.e. the DNA  
78 STRIP technology GenoType LeptraeDR<sup>®</sup> Hain Lifescience) [7] or whole-genome sequencing  
79 (WGS). [8] Because of the complexity of the phenotypic method, genotypic methods are  
80 currently the main methods used to diagnose AMR in *M. leprae*. Reliable data concerning  
81 mutations and their impact on AMR are required, especially since the presence of a mutation in  
82 a gene encoding a drug target or an activator does not necessarily confer resistance. [9]  
83 In this review, we focused on the fluoroquinolones (FQ) since (i) they are effective and  
84 powerful bactericidal drugs against *M. leprae* [10–12] and (ii) their use for the treatment of  
85 other infections has promoted the emergence of resistance, as in the case of *M. tuberculosis*.  
86 [13,14] According to the first global resistance data published in 2018, with resistance to FQ  
87 diagnosed using genotypic methods, 1,33% of 1581 *M. leprae* isolates studied were resistant to  
88 ofloxacin. [5] FQ targets are generally the type II topoisomerases (i.e. DNA gyrase and  
89 topoisomerase IV), but with *M. leprae* lacking topoisomerase IV, the DNA gyrase is the sole  
90 target of FQ in this organism. [8]  
91 The purpose of our review was (i) to characterize all DNA gyrase gene mutations described in  
92 clinical strains of *M. leprae* and (ii) to distinguish between those associated with FQ resistance  
93 and those associated with susceptibility. The existing tool for this latter purpose was a model

94 of the cleavage core of *M. leprae* gyrase. [15] Therefore, we aimed to develop further the model  
95 by building a 3D model of the full-length *M. leprae* gyrase enabling to evaluate the impact of  
96 mutations whose role in FQ resistance has not been demonstrated previously whatever their  
97 location in the DNA gyrase sequence. This review summarizes all substitutions described in  
98 GyrA and GyrB in clinical strains of *M. leprae*. It also includes the first proposal of a consensus  
99 numbering system for *M. leprae* GyrA and GyrB which should allow a standardized comparison  
100 of all mutations reported.

101

## 102 **METHODS**

103

### 104 **Definitions**

105 Mutation was indicated as a base-pair change that led to an amino acid substitution, irrespective  
106 of whether the mutation occurred in a FQ-resistant or a FQ-susceptible *M. leprae* isolate.  
107 Among the mutations, we distinguished between those found to confer FQ resistance, in  
108 biochemical experiments or on the basis of clinical and epidemiological criteria, and those  
109 apparently unrelated to resistance.

110 Biochemical method is an *in vitro* technique enabling to evaluate the impact of DNA gyrase  
111 mutations on FQ efficacy by measuring the FQ concentrations required to inhibit the DNA  
112 supercoiling activity of the DNA gyrase (IC<sub>50</sub>) (Table 5). Comparing the FQ concentrations  
113 needed for the WT *M. leprae* DNA gyrase and the mutated enzymes enables to evaluate the  
114 impact of mutations of DNA gyrase on susceptibility to FQ. These data correlate with *in vivo*  
115 efficacy of FQ. [16]

116 Polymorphism was indicated as non-synonymous nucleotide base-pair changes known not to  
117 be associated with, or not to confer, FQ resistance. We did not include the base-pair changes  
118 that did not result in an amino acid change, i.e. synonymous mutation. We used the three-letter

119 abbreviation nomenclature for amino acids: substitutions were indicated as Xxx##Yyy, where  
120 Xxx was the wild-type amino acid, ## the codon number (and by the same token the amino acid  
121 number) and Yyy the substituting amino acid.

122

### 123 **Research methodology**

124 A bibliographic research was used to identify peer-reviewed primary studies reporting FQ-  
125 resistant and -susceptible isolates of *M. leprae*, or isolates without documented drug  
126 susceptibility (i.e phenotypic drug susceptibility testing by using the mouse footpad model) in  
127 which mutations in DNA gyrase genes were identified. We limited the research to studies  
128 published between January 1, 1990 and December 27, 2020. Full-text articles were screened  
129 using the Medical Literature Analysis and Retrieval System Online (MEDLINE) with the  
130 keywords '*M. leprae*', 'leprosy', 'fluoroquinolone resistance', 'fluoroquinolone susceptibility',  
131 'DNA gyrase', 'GyrA', 'GyrB', 'mutation', 'substitution', 'drug resistance', 'antimicrobial  
132 resistance', 'ofloxacin resistance' and 'ofloxacin susceptibility' in different combinations.  
133 Figure S1 shows the study selection procedure (supplementary data).

134 The inclusion criteria called for publications that reported (i) genotypic susceptibility-testing  
135 methods and (ii) DNA gyrase gene mutations identified in *M. leprae* DNA obtained from  
136 human clinical specimens.

137 We excluded publications if they were reviews and duplicates, or if the title indicated that the  
138 study was not relevant to our review. We reviewed the abstracts of the remaining papers and  
139 we excluded studies with irrelevant content. The entire article was reviewed before exclusion  
140 only if the abstract did not provide enough information to include or exclude the article. Articles  
141 with no data on amino acid changes were also excluded.

142

### 143 **Data acquisition**



144 We organized the data abstracted from journal articles that met the inclusion criteria in three  
145 groups: all mutations reported (i) in *gyrA*, or (ii) in *gyrB*, and (iii) all combinations of mutations  
146 (in *gyrA* and/or *gyrB*) reported in a single *M. leprae* isolate. When more than one mutation was  
147 observed in one strain, we considered two scenarios: (i) each mutation was observed as a single  
148 mutation elsewhere or (ii) the mutations were never observed independent of one another. In  
149 both scenarios, the mutations were listed as single mutations and as multiple mutations. This  
150 process was designed to record every mutation without failing to appreciate the potential effect  
151 that combinations of mutations may have on FQ resistance.

152 The number of isolates taken into account in each study corresponds to the number of isolates  
153 for which the DNA gyrase sequence (independent of the technique used) was available.  
154 Moreover, when an isolate was already described in a publication, it was counted only once.

155 Regarding the numbering system, some authors used the one of *Escherichia coli*, others used  
156 the *M. leprae* numbering system for the location of substitutions, while still others did not  
157 mention which system they used. [17] In this systematic review, all substitutions in GyrA are  
158 given based on the standardized *M. leprae* genome numbering system [18], and all substitutions  
159 in GyrB based on the re-annotated gene numbering system of *M. tuberculosis* GyrB. [19]

160 We report the number of clinical isolates tested, the region sequenced (entire *gyrA* or *gyrB* or  
161 only the Quinolone-Resistance Determining Region (QRDR) of *gyrA* and/or *gyrB*) as well as  
162 the methods (genotypic or phenotypic) used to determine FQ susceptibility in each study. The  
163 number of isolates containing a specific mutation is given, along with the phenotypic FQ  
164 susceptibility profile and the prior history of FQ use, associated with the mutation if reported.  
165 FQ activity (measured as the 50% inhibitory concentration) against *M. leprae* DNA gyrase with  
166 specific mutations was also reviewed.

167

168 **Modeling**

169 Template-based protein structure was predicted using Protein Homology/AnalogY Recognition  
170 Engine 2 (Phyre2; [www.sbg.bio.ic.ac.uk/phyre2/](http://www.sbg.bio.ic.ac.uk/phyre2/)). [20] The chosen model was based on the 3D  
171 structure of the full-length gyrase of *E. coli* recently obtained by cryo-EM (PDB code 6RKW).  
172 [21] Briefly, the intein region stretching from residue positions 131-551 were removed before  
173 modeling. The modelled region of chain A corresponds to sequence numbers 016-130 and 552-  
174 1241 and the chain B is modelled from residue numbers 008-663. Quality of the build model  
175 was estimated by ProQ2 (implemented in Phyre2). [22] The inhibitory molecule presents in the  
176 chosen model is Gepotidacin and not a fluoroquinolone. Thus, to complete our model of *M.*  
177 *leprae* gyrase in complex with DNA and moxifloxacin we removed Gepotidacin and introduced  
178 the two FQ moieties extracted from the X-ray structure of the *M. tuberculosis* gyrase cleavage  
179 core in complex with dsDNA and moxifloxacin (PDB code 5BS8). [23] The two FQ moieties  
180 were positioned in the cleavage core with respect to their respective positions in the 5BS8  
181 structure.

182

### 183 **Quality control**

184 Four authors (A.C., F.R., F.M. and A.A.) independently reviewed and abstracted the data. One  
185 author (E.C.) also reviewed the data for accuracy and adjudicated differences among  
186 publications. C.M. proposed a consensus numbering system and S.P. performed the modeling  
187 of DNA gyrase carrying substitutions. All authors participated in the writing of the manuscript.

188

## 189 **RESULTS**

190

### 191 **Numbering system for the *M. leprae* GyrA and GyrB subunits**

#### 192 **GyrA**

193 The studies that investigated the molecular basis of FQ resistance of *M. leprae* were mainly  
194 based on *M. leprae* gene sequences, [13,24–26] and rarely on *E. coli* sequences. [27,28] Since  
195 the QRDR of GyrA is located at the N-terminal part of the GyrA subunit and the *M. leprae*  
196 *gyrA* start codon is eight and one codon(s) upstream of those of *E. coli* and *M. tuberculosis*,  
197 respectively (Figure 1), the numbers of the amino acid positions change according to the  
198 numbering system used. For *M. leprae*, the QRDR of GyrA therefore ranges from positions 75  
199 to 114, for *E. coli* from 67 to 106 and for *M. tuberculosis* from 74 to 113 (Table 1 and Figure  
200 1).

201 A recent study used WGS to identify SNPs involved in AMR in *M. leprae*. [29] In contrast to  
202 other studies using PCR sequencing or a line probe assay, WGS allows the analysis of the entire  
203 *gyrA* gene, including the intein-encoding 1260-base-pair sequence inserted into *gyrA* near the  
204 codon for the active-site tyrosine (Figure 1). As this intein is removed during splicing, [30] we  
205 propose that future studies use the numbering system based on the alignment of the *M. leprae*  
206 GyrA subunit with the *E. coli* and *M. tuberculosis* GyrA subunits, not taking into account the  
207 intein. A specific numbering system is proposed for the intein, starting from 1 to 420 as  
208 GyrA\_intein Xxx##Yyy.

## 209 **GyrB**

210 In Table 2, we propose a consensus numbering system for *M. leprae* aligned with the three  
211 *M. tuberculosis* numbering systems described for GyrB [28] and the *E. coli* numbering system.  
212 The GyrB QRDR stretches from amino acid 426 to 464, from 461 to 499 and from 464 to 502  
213 in *E. coli*, *M. tuberculosis* and *M. leprae*, respectively (Figure 2), while an extension of the  
214 *M. tuberculosis* QRDR to amino acid 501 has been proposed previously. [31]

## 215 **Findings**

216 Twenty-five publications met our inclusion criteria. In these studies, 2884 clinical *M. leprae*  
217 isolates were assessed for genotypic analyses. Most of the strains were isolated from patients  
218 with multibacillary infections (75%) and from patients with relapses (37%), but corresponding  
219 information was missing in seven studies (Table 3).

220 In twenty studies only the QRDR of *gyrA* was sequenced, in four the QRDR of both *gyrA* and  
221 *gyrB* and in one the whole genome (Table 3). Amino acid substitutions in GyrA were found in  
222 110 clinical isolates whereas substitutions in GyrB were identified in six clinical isolates.  
223 Specific substitutions identified in GyrA and GyrB are described in the following sections and  
224 in Table 4. Among the 2884 clinical isolates studied, 21 distinct substitutions were identified  
225 and concerned 17 different codons, with 18 substitutions in GyrA and three in GyrB.

226 Only three studies reported testing of the phenotypic susceptibility of *M. leprae* to FQ using the  
227 mouse footpad method and found that the three FQ-resistant isolates they studied carried an  
228 A91V substitution in GyrA. [13,25,26]

229 The prior use of FQ was reported in 18 studies (Table 4). [13,24–26,29,32–44]

230

### 231 **Mutations in *gyrA*** (Tables 1, 4)

232 Among the 18 substitutions described in GyrA, nine were inside and seven outside the QRDR,  
233 and two were inside the intein (Table 1). The A91 substitution was the most prevalent. It was  
234 found in 75 (68%) of the 110 clinical strains that harbored a substitution in GyrA. Three  
235 different substitutions were reported at this position, A91V, A91T and A91P. The A91V  
236 substitution was the most prevalent (70, 3 and 2 strains harbored A91V, A91T and A91P  
237 substitutions, respectively). Interestingly, for the three strains carrying the A91V substitution  
238 that were tested, the phenotypic mouse footpad method confirmed the diagnosis of resistance  
239 to FQ. [13] Of the patients carrying isolates with other substitutions in GyrA, 10 had a relapse

240 and one was under FQ treatment while none reported previous use of FQ (Table 4). No  
241 differences exist in the occurrence of *gyrA* mutants by region/ origin.

242 Three strains with substitutions in GyrA also harbored a substitution in GyrB while *gyrB* was  
243 not sequenced in all the studies. Multiple substitutions in GyrA were found in three strains  
244 (Table 4).[29,35,41] Double mutations in *gyrA* have been described and associated with high-  
245 level resistance. [45] They may result from a two-step selection of FQ-resistant mutants, which  
246 unlikely occurs in the extremely slow growing *M. leprae*.

247

#### 248 **Mutations in *gyrB*** (Tables 3, 4)

249 Only five studies reported substitutions in GyrB, using PCR sequencing or WGS.[3,29,42–44]  
250 Among the 755 strains studied for GyrB, only nine (1.2%) harbored substitutions corresponding  
251 to three amino acid changes. Among these, two were inside the QRDR (D464N, also named  
252 D205N by two authors, and T503I) [3,29,44] and one was outside (V214G) [29,43] (Table 4).  
253 The phenotypic method was not performed for any strain. Two patients carrying isolates with  
254 a substitution in GyrB had a relapse, one of whom reported previous use of FQ. [3,44] Three  
255 strains with substitutions in GyrB also harbored a substitution in GyrA. [29] Multiple  
256 substitutions in GyrB were not found.

257

#### 258 **Impact on susceptibility to FQ**

259 We focused on mutations that conferred FQ resistance rather than those present in FQ-resistant  
260 *M. leprae* isolates (*i.e.* on mutations for which biochemical studies demonstrated that the  
261 modified DNA gyrase subunit was resistant to FQ inhibition) (Table 5) as well as on modeling,  
262 which enables the prediction of possible impacts of unstudied mutations on FQ susceptibility  
263 (Figure 3). [7,13,17,24–26] We have shown that residues at positions 87, 89, 91 and 92 in GyrA  
264 and 464, 503 in GyrB (all in the QRDR and following the proposed consensus numbering

265 system) are localized in the close vicinity of the binding site of the drug. Consequently, their  
266 substitution could impact the binding of the drug (Figure 3A) and thus the FQ susceptibility of  
267 *M. leprae*. Concerning the residue 107 in the QRDR of GyrA, it is also in the vicinity of the  
268 bound drug but not close enough for us to assert that it can impact the binding of the drug  
269 (Figure 3A). Residues at position 311 and 431 in GyrA and 214 in GyrB are located at the  
270 domains' interfaces of the protein, in the breakage-religation domain and the ATPase domain  
271 of GyrA and GyrB, respectively (Figure 3B-C). They might play a role in the conformational  
272 movements of the protein, but their impact on FQ binding is unpredictable. A role in FQ binding  
273 can also not be predicted for residue 695 in GyrA since it is located in the C-terminal domain  
274 (CTD) and is therefore implicated in the binding of the DNA to the CTD, i.e. far from the FQ-  
275 binding pocket (Figure 3B).

276 Table 5 lists the DNA gyrase substitutions that have been demonstrated to confer FQ resistance  
277 in *M. leprae* based on this gyrase modeling.

278

## 279 **DISCUSSION**

280 During the review process of mutations in *M. leprae* DNA gyrase associated with FQ-  
281 resistance, discrepancies generated confusion hampering their identification as previously  
282 described or new mutations. Therefore, as the first mechanism of FQ resistance in *M. leprae*  
283 involves various amino acid changes in the DNA gyrase, we considered it useful (i) to propose  
284 a consensus numbering system for the unambiguous identification of the gyrase mutations  
285 (Figures 1 and 2, Table 1) and (ii) to apply it to all substitutions in the GyrA and GyrB subunits  
286 described to date.

287 Our review includes the mutations reported inside and outside the QRDR, including those in  
288 the intein of GyrA, and notes which have been reported to confer resistance. We carefully  
289 reviewed the literature to use the correct denominator and not to count identical isolates more

290 than once. Indeed, since in the individual publications the number of interpretable *gyrA* and/or  
291 *gyrB* sequences was often smaller than that of the strains studied, such caution seemed  
292 particularly warranted.

293 The main limitation of our work relates to the limitations of the most widely used molecular  
294 method to detect FQ resistance in *M. leprae*. Indeed, we have shown that PCR sequencing of  
295 the *gyrA* QRDR was the most widely used whereas PCR sequencing of the *gyrB* QRDR was  
296 rarely performed (Table 3). The line probe assay GenoType LeptraeDR<sup>®</sup> used in only two  
297 studies has also limitations. [37,39] Despite the fact that the line probe assay allows detection  
298 of the most important mutations in the three main genes involved in antibiotic resistance in  
299 *M. leprae* (i.e. *rpoB*, *folP* and *gyrA*), its weakness is that it focuses on the QRDR of only *gyrA*,  
300 and then on codon 91 directing the A91V substitution, while other mutations in this region  
301 require verification by PCR sequencing. This latter detail could explain that among *M. leprae*  
302 strains harboring mutations in DNA gyrase, only 5% had mutations in *gyrB* (Table 4), whereas  
303 in *M. tuberculosis* they are responsible for FQ resistance in 10% of clinical strains. [28] As in  
304 the study using WGS 23% of the strains were found to carry a mutation in *gyrB*, we could  
305 expect that using methods enabling the detection of mutation in the entire *gyrA* and *gyrB* genes  
306 would allow the detection of more mutations in *gyrB*. [29] Consequently, the role of *gyrB*  
307 mutations in FQ resistance in leprosy remains difficult to assess, but despite their apparent  
308 rarity, mutations in *gyrB* should be searched for systematically in drug resistance screening.  
309 [29,46]

310 With the emergence of the WGS techniques, mutations outside the QRDR are more often found,  
311 increasing the need for a unique numbering system for both GyrA and GyrB. For substitutions  
312 in GyrA, we propose a numbering system based on the alignment of *M. leprae* for GyrA QRDR  
313 between codons 75 and 114, not taking into account the intein, and a numbering system specific  
314 to the intein between codons 1 and 420 (Figure 1, Table 1). Since the intein is excised after

315 transcription, it would be confusing to consider intein mutations in the context of substitutions  
316 in GyrA. For GyrB substitutions, we propose a numbering system based on the reference system  
317 for *M. tuberculosis* GyrB published by Camus *et al.* (Figure 2, Table 2). [47]

318 In GyrA, the A91V substitution was the most frequently encountered and occurred in 75 of 110  
319 strains. In a smaller proportion, other substitutions were found in clinical strains within the  
320 QRDR of GyrA (e.g. P87L, G89C, A91P, A91T, S92A and R107L) outside the QRDR (V311I,  
321 I431T, G362E and G695R), and within the intein (S177L and G232E) (Table 1). Regarding  
322 GyrB, three different substitutions were reported. They were labelled V214G, D464N and  
323 T503I by the authors, but the D464N substitution, the most frequently described, was also  
324 labelled D205N, which illustrates the crucial need for unification of the numbering systems  
325 (Tables 2 and 4).

326 Evaluating drug susceptibility of *M. leprae* is challenging and requires the cumbersome mouse  
327 footpad technique since this pathogen does not grow *in vitro*. [48] Biochemical studies are of  
328 help to reliably predict the impact of DNA gyrase substitutions on FQ resistance. [9,16,18,49]  
329 Consistent with biochemical studies (Table 5), GyrA A91V and G89C substitutions were shown  
330 to be resistant to FQ by using the phenotypic method.[13,18,50]

331 A possible involvement of the other substitutions in GyrA found in clinical *M. leprae* isolates  
332 in the susceptibility to fluoroquinolones has still to be explored (i.e. of P87L, A91P, A91T,  
333 S92A, R107L, V311I, I431T and G695R in GyrA and of S177L and G232E in the intein).  
334 Thanks to the modeling, we observed that FQ binding occurs in the vicinity of GyrA residues  
335 87, 89, 91 and 92. Thus, the proline to leucine substitution at position 87 induces a size reduction  
336 in the binding cavity through the presence of a longer hydrophobic side chain. For the  
337 substitution of alanine 91 by threonine or proline, the same effect is observed, in addition to the  
338 effect of a polar group. Inversely, the serine 92 to alanine substitution induces an enlargement



339 of the binding cavity. Concerning the other GyrA substitutions, our model predicts that, even if  
340 the arginine 107 to leucine substitution leads to a drastic charge modification for a binding  
341 cavity, its role in FQ susceptibility cannot be predicted due to its great distance from FQ binding  
342 sites. Based on our model, we can predict that the GyrA V311I, I431T and G695R substitutions  
343 are not implicated in FQ resistance.

344 As for GyrB, the implication of only the D464N substitution in FQ resistance has been  
345 demonstrated unequivocally. [18,50] Regarding the substitution called D205N, it appears, after  
346 careful review of the papers that reported strains harboring it, [3,44] that it was mislabeled and  
347 is actually equivalent to the D464N substitution. Since, we did not notice previously that these  
348 substitutions are identical and since there exists an aspartic acid at position 205 in a sequence  
349 that was later deleted from the databases, we have generated a mutated *M. leprae* gyrase  
350 harboring the “true” D205 that was, not surprisingly, found not to be implicated in FQ resistance  
351 in biochemical assays. [18] This was, however, the case of the “true” D464N (Table 5). [50]  
352 Interestingly, the corresponding patients were previously treated with FQ. Regarding the other  
353 GyrB substitutions (Tables 2, 4 and 5), no information was available regarding previous  
354 treatment of the patients, but the modeling suggests that they are not implicated in FQ resistance  
355 since they are not located in the FQ-binding pocket (Figure 3).

356 Despite resistance to the other two main antileprosy drugs (i.e rifampin and dapsone) occurred  
357 in some of the FQ-resistant strains, leading to multi-drug resistance, we did not review  
358 information regarding *rpoB* and *folP* mutations in our work since studying resistance to all  
359 antileprosy drugs, or multidrug resistance, was not under the scope of this review.

360 Currently, analyzing FQ resistance exclusively by sequencing of the QRDR of *gyrA* may lead  
361 to a possible underestimation of FQ resistance in leprosy. While WGS appears to be the most  
362 adequate approach to the comprehensive identification of mutations implicated in FQ

363 resistance, complementary biochemical studies will be required to determine their precise role  
364 in the loss of susceptibility. The consensus numbering system proposed here for substitutions  
365 in GyrA and GyrB (with consideration of those occurring within the intein) should allow for  
366 straightforward comparison of sequence data from resistant *M. leprae* isolates.

367

368

369 **Transparency declaration**

370 All authors declare no financial relationships with any organizations that might have an interest  
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373

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378 **Author contributions**

379 AA designed the research. AC, FM, FR, SP, CM, EC and AA conducted the research. AC wrote  
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381 All authors contributed to the data interpretation, revised each draft for important intellectual  
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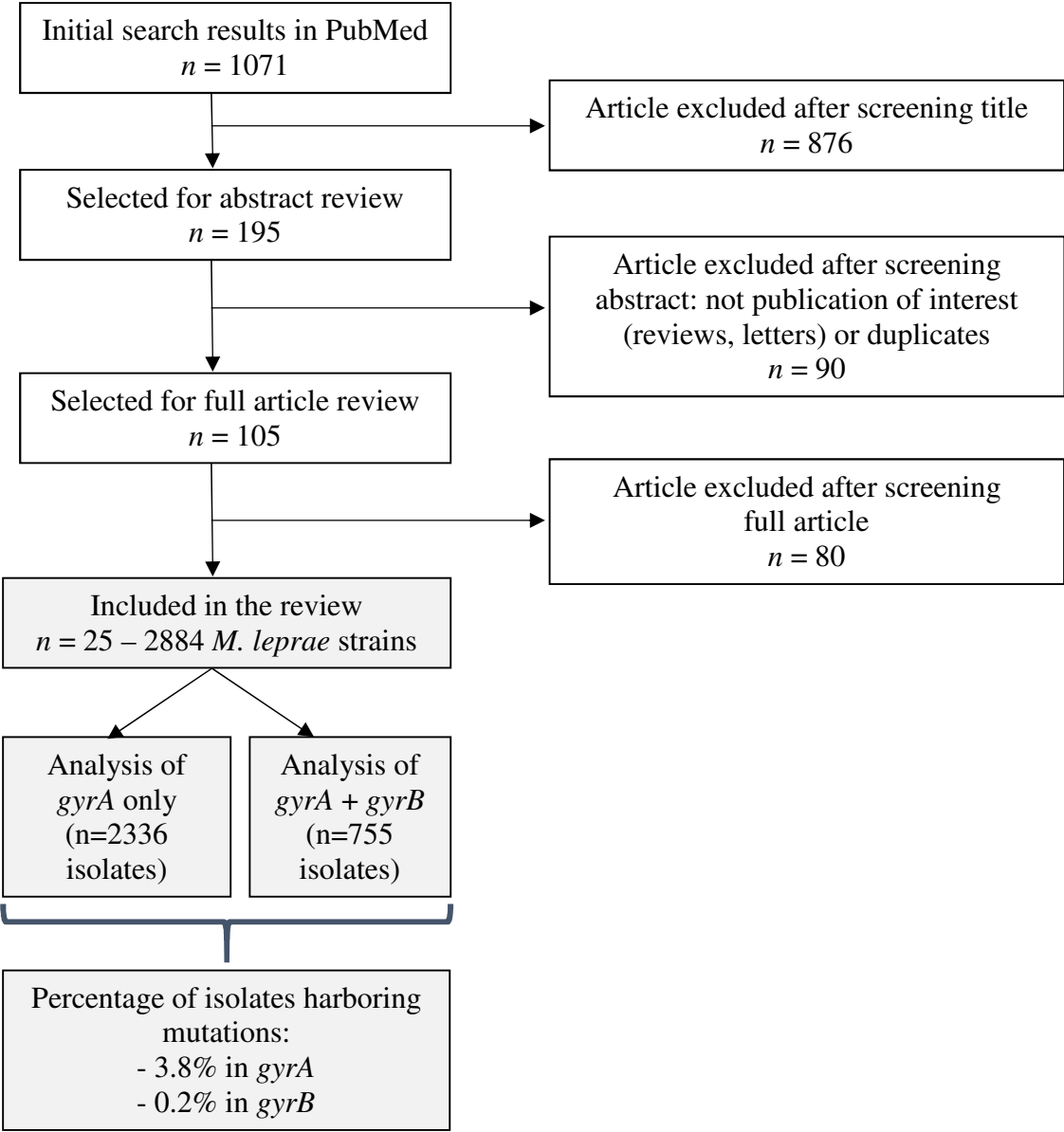
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559

**Graphical abstract.** Flow diagram summarizing the literature selection and main results.





sp	P0AES4	GYRA_ECOLI	IAVGMATNIPPHNLTEVINGCLAYID----DEDISIIEGLMEHIPGPDFPTAAIINGRRGI	229
sp	P9WG47	GYRA_MYCTU	IAVGMATNIPPHNLRELADAVFWALENHDADEEETLAAVMGRVKGPDFPTAGLIVGSQGT	240
sp	Q57532	GYRA_MYCLE	IAVGMATNIPPHNLYELADAVFWCLENHDADEETMLVAVMERVKGPDFPTAGLIVGSQGI	241
			*****μ*: : : : μ*: : : : **: μμ: μ*: *μ*: : *****: : **: : *μ	
sp	P0AES4	GYRA_ECOLI	EEAYRTGRGKVYIRARAEVEVDAKTGRETIIIVHEIPYQVNKARLIEKIAELVKEKRVEGI	289
Sp	P9WG47	GYRA_MYCTU	ADAYKTGRGSIRMRGVVEVEEDSR-GRTSLVITELPYQVNHDNFITSIAEQVRDGKLAGI	299
Sp	Q57532	GYRA_MYCLE	ADAYKTGRGSIRIRGVVEVEEDSR-GRTSLVITELPYQVNHDNFITSIAEQVRTGRLAGI	300
			: : **: *****: : μ*: : : **: *: : : **: : : : **: *****: : : **: : **: *μ: μ: : * *	
sp	P0AES4	GYRA_ECOLI	SALRDES-DKDGMRIVIEVKRDAVGEVVLNLYSQTQLQVSFGINMVALHHGQPKIMNLK	348
sp	P9WG47	GYRA_MYCTU	SNIEDQSSDRVGLRIVIEIKRDAVAKVVINLNLYKHTQLQTSFGANMLAIVDGVPTLRLD	359
sp	Q57532	GYRA_MYCLE	SNVEDQGS DRVGVRIVIEIKRDAVAKVVLNLYKHTQLQTSFGANMLSIVDGVPTLRLD	360
			*: μ*: *μ*: *: : *μ*****: *μ*****: *****: *****: *μ: : : **: : : : *:	
sp	P0AES4	GYRA_ECOLI	DIIAAFVHRHREVVTRRTIFELRKARDRAHILEALAVALANIDPIIELIRHAPTPAEAKT	408
sp	P9WG47	GYRA_MYCTU	QLIRYYVDHQLDVIVRRTTYRLRKANERAHILRGLVKALDALDEVIALIRASETVDIARA	419
sp	Q57532	GYRA_MYCLE	QMICYVEHQLDVIVRRTTYRLRKANERAHILRGLVKALDALDEVITLIRASQTVDIARV	420
			: μ*μ*: *μ*: : *: : **: : : **: : : **: : : *: : *μ****: μ*: : : *: μ	
sp	P0AES4	GYRA_ECOLI	ALVANPWQLGNVAAMLERAGDDAARPEWLEPEFGVRDGLYYL TEQQAQAILDLRLQKLTG	468
sp	P9WG47	GYRA_MYCTU	GLIE-----LLDIDEIQQAAILDMQLRRLAA	445
sp	Q57532	GYRA_MYCLE	GVVE-----LLDIDDIQAQAILDMQLRRLAA	446
			: μμ: : : : : : : : : : : : : : : : *: : : μ: *****: **: *:	
sp	P0AES4	GYRA_ECOLI	LEHEKLLDEYKELLDQIAELLRILGSADRLMEVIREELELVREQFGDKRRTEITANSADI	528
sp	P9WG47	GYRA_MYCTU	LERQRIIDDLAKIEAEIADLEDILAKPERQRGIVRDELAEIVDRHGDDRRTRIIAADGDV	505
sp	Q57532	GYRA_MYCLE	LERQRIIDDLAKIEVEIADLGILAKPERRRGI I RNELTEIAEKYGDDRRTRIIAVDGDV	506
			** : : : : * : : : : μ: * *: *μ: * *: : : *μ: : : μ*μ**μ: : μμμ** : * *: * : *μ: : *:	



**Figure 2.** Sequence alignment of the GyrB subunit of *E. coli* (P0AES6), *M. tuberculosis* (P9GW45) and *M. leprae* (Q59533). The numbering system of *M. tuberculosis* P9GW45 is the proposed consensus numbering system for the GyrB subunit.

\* = identical residue between *E. coli*, *M. tuberculosis* and *M. leprae*, : = identical residue between *M. tuberculosis* and *M. leprae* but different from *E. coli* and  $\mu$  = non-conserved substitution between *M. tuberculosis* and *M. leprae*. The QRDR is highlighted in grey. The start codons are in bold.

sp	P0AES6	GYRB_ECOLI	----- <b>M</b> NSYDSSSIKVLKGLDAVRKRPGMYIGD	TDDGTGLH	HMVFEVVDNAIDEALA	53	
sp	P9GW45	GYRB_MYCTU	<b>M</b> AAQKKKAQDEYGAASITILEGLEAVRKRPGMYIGSTGE	-RGLHHLIWEVVDNAVDEAMA		59	
sp	Q59533	GYRB_MYCLE	<b>M</b> AAQR-KAQDEYGAASITILEGLEAVRKRPGMYVGSTGE	-RGLHHLIWEVVDNSVDEAMA		58	
			$\mu$ :: : $\mu$ :: : : * :: : * * :: : : * * : * * * * * * * * $\mu$ * : * :: : : * * * * : : : * * * * * $\mu$ : * * * : *				
sp	P0AES6	GYRB_ECOLI	GHCKEIIIVTIHADNSVSVQDDGRGIPTGIHPEEGVSAAEVIMTVLHAGGKFDD	--NSYKV		111	
sp	P9GW45	GYRB_MYCTU	GYATTVNVVLLLEDGGVEVADDGRGIPVATHAS-GIPTVDVVMTQLHAGGKFDS	--DAYAI		116	
sp	Q59533	GYRB_MYCLE	GYATQVDVRLFDDGSVEVADNGRGIPVAVHAT-GVPTVDVVMTQLHAGGKFGGKDSGYNV			117	
			* :: : $\mu$ : $\mu$ * $\mu$ : $\mu$ * $\mu$ * : $\mu$ * : * : * $\mu$ * * * * * : : $\mu$ * : $\mu$ : * $\mu$ : : : : * : * * : * * * * * * * $\mu$ $\mu$ $\mu$ $\mu$ $\mu$ * $\mu$ $\mu$				
sp	P0AES6	GYRB_ECOLI	SGGLHGVGVSVVNALSQKLELVIQREGKIHRQIYEHGVPQAPLAVTGETEKTGTMVRFWP			171	
sp	P9GW45	GYRB_MYCTU	SGGLHGVGVSVVNALSTRLEVEIKRDGYEWSQVYEKSEPLG-LKQGAPTKKTGSTVRFWA			175	
sp	Q59533	GYRB_MYCLE	SGGLHGVGVSVVNALSTRVEVDIKRDGYEWSQFYDKAVPGI-LKQGEATEATGTTIRFWA			176	
			* * * * * * * * * * * * * * * * : : $\mu$ * : $\mu$ * : * : * : : : * $\mu$ * $\mu$ : $\mu$ $\mu$ * $\mu$ $\mu$ : * : : : $\mu$ * $\mu$ $\mu$ * * $\mu$ : $\mu$ * * * :				
sp	P0AES6	GYRB_ECOLI	SLETFTNVTEFEYEILAKRLRELSFLNSGVSIRLRDKRDGKED-----			214	
sp	P9GW45	GYRB_MYCTU	DPAVFE-TTEYDFETVARRLQEMAFLNKGLTINLTDERVTQDEVVDEVSDVAEAP--KS			232	
sp	Q59533	GYRB_MYCLE	DPDIFE-TTKYDFGTVARRIQEVAFLNKGLTINLVDERVKQDEVVDDVVSDTAEAPVAMT			235	
			: : $\mu$ $\mu$ * : : : * $\mu$ : : : $\mu$ : : * : * $\mu$ : * $\mu$ : * * * : * : : * : * $\mu$ * : * : $\mu$ : : : : : $\mu$ : : : : : $\mu$ : : : : : $\mu$ $\mu$ $\mu$ $\mu$				

sp | P0AES6 | GYRB\_ECOLI | -----HFHYEGGIKAFVEYLNKNKTP IHPNIFYFSTEKDGIGVEVALQ 257  
sp | P9GW45 | GYRB\_MYCTU | ASERAAESTAPHKVKSRTFHYPGGLVDFVKHINRTKNAIHSSIVDFSGKGTGHEVEIAMQ 292  
sp | Q59533 | GYRB\_MYCLE | VEEKSTESSAPHKVRHRTFHYPGGLVDFVKHINRTKTP IQQSIIDFDGKGAGHEVEVAMQ 295  
μμ:μμμ:μ:::μμ:\*\*\*:\*\*\*::\*\*::\*:\*\*μμ\*μμ:\*μ:\*μ::μ\*:\*\*μ\*:\*

sp | P0AES6 | GYRB\_ECOLI | WNDGFQENIYCFTNNIPQRDGGTHLAGFRAAMTRTLNAYMDKEGYSKKAKVSATGDDARE 317  
sp | P9GW45 | GYRB\_MYCTU | WNAGYSESVHTFANTINTHEGGTHEEGFRSALT SVVNKYAKDRKLLKDKDPNLTGDDIRE 352  
sp | Q59533 | GYRB\_MYCLE | WNGGYSESVHTFANTINTHEGGTHEEGFRSALT SVVNKYAKDKLLKDKDPNLTGDDIRE 355  
\*\*μ\*::\*:::~\*:\*:\*:::~\*\*\*\*::~\*\*\*:\*:\*:::~\*:\*::μ::\*:::~\*\*\*\*:\*~

sp | P0AES6 | GYRB\_ECOLI | GLIAVVSVKVPDPKFSSQTKDKLVSSEVKS SAVEQQMNELLA EYLL ENPTDAKIVVGKI ID 377  
sp | P9GW45 | GYRB\_MYCTU | GLAAVISVKVSEPQFEGQTKTKLGNTEVK SFVQKVCNEQL THWF EANPTDAKVVVNKA VS 412  
sp | Q59533 | GYRB\_MYCLE | GLAAVISVKVSEPQFEGQTKTKLGNTEVK SFVQ RVCNEQL IHWFEANP VDAKAVVNKA IS 415  
\*:\*\*:\*~\*\*\*::~\*:\*::~~\*\*\*:\*~\*:::~\*\*\*\*:\*~μ::\*\*:\*~μ:::~\*\*μ\*\*\*μ\*\*::~\*~μ:

sp | P0AES6 | GYRB\_ECOLI | AARAREAAARRAREMTRRK GALDLAGLPGKL ADCQERDPALSELYLVEGDSAGGSAKQGRN 437  
sp | P9GW45 | GYRB\_MYCTU | SAQARIAARKARELVRRKSATDIGGLPGKL ADCRSTDPRKSELYVVEGDSAGGSAKSGRD 472  
sp | Q59533 | GYRB\_MYCLE | SAQARIAARKARELVRRKSATDIGGLPGKL ADCRSTDPRSSELYVVEGDSAGGSAKSGRD 475  
:\*:\*\*:\*~\*\*\*::~\*:\*::~~\*\*\*:\*~\*~μ:~\*\*\*\*\*:::~\*\*~μ\*\*\*\*\*~\*\*\*::~\*~:

sp | P0AES6 | GYRB\_ECOLI | RKNQAILPLKGKILNVEKARFDKMLSSQEVATLITALGCGIGRDEYNPDKLRYHSIIIMT 497  
sp | P9GW45 | GYRB\_MYCTU | SMFQAILPLRGKI INVEKARIDRVLKNTEVQAIITALGTGIH-DEFDIGKLRYHKIVLMA 531  
sp | Q59533 | GYRB\_MYCLE | SMFQAILPLRGKI INVEKARIDRVLKNTEVQAIITALGTGIH-DEFDISRLRYHKIVLMA 534  
:::~\*\*\*\*\*:\*~\*\*\*:~\*\*\*\*\*::~\*:\*::~~\*\*\*::~~\*\*\*\*\*:\*~\*\*::~μμ\*\*\*\*\*::~\*~\*~:



sp | P0AES6 | GYRB\_ECOLI | DADVDGSHIRLLLLTFFYRQMPEIVERGHVYIAQPPLYKVKKGKQEYIKDDEAMDQYQI | 557  
SP | P9GW45 | GYRB\_MYCTU | DADVDGQHISTLLLLTLLFRFMRLIENGHVFLAQPPLYKWKQRSDPEFAYS DRERDGLL | 591  
sp | Q59533 | GYRB\_MYCLE | DADVDGQHISTLLLLTLLFRFMRLIEHG YVFLAQPPLYKWKQRMDPEFAYS DSERDGLL | 594  
\*\*\*\*\*: \*\* :\*\*\*\*\* : : \* : \* : : : \* \* \* μ \* : \* \* \* \* \* \* \* : \* : : μ : : : : : : : : : : : : : : : :

sp | P0AES6 | GYRB\_ECOLI | SIALDGATLHTNASAPALAGEALEKLVSEYNATQKMINRMERRY PKAMLKELIYQPTLTE | 617  
SP | P9GW45 | GYRB\_MYCTU | EAGLKAG----- | 598  
sp | Q59533 | GYRB\_MYCLE | ETGLKLG----- | 601  
: μ : \* : μ :

sp | P0AES6 | GYRB\_ECOLI | ADLSDEQTVTRWVNALVSELNDKEQHGSQWKFDVHTNAEQNLFEPIVRVRTHGV DTDYPL | 677  
SP | P9GW45 | GYRB\_MYCTU | ----- | 598  
sp | Q59533 | GYRB\_MYCLE | ----- | 601  
: :

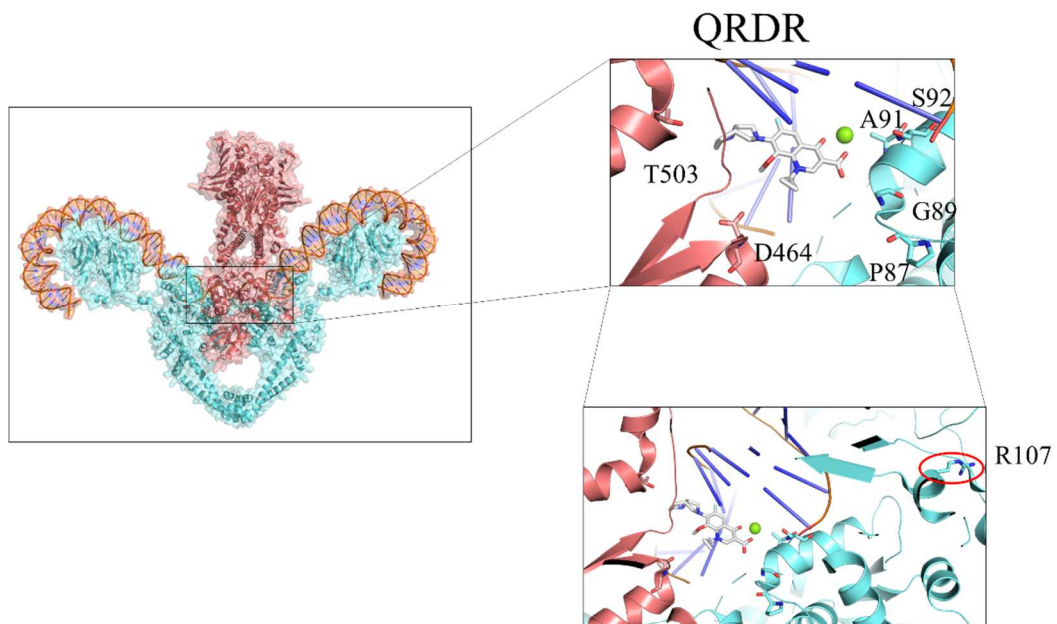
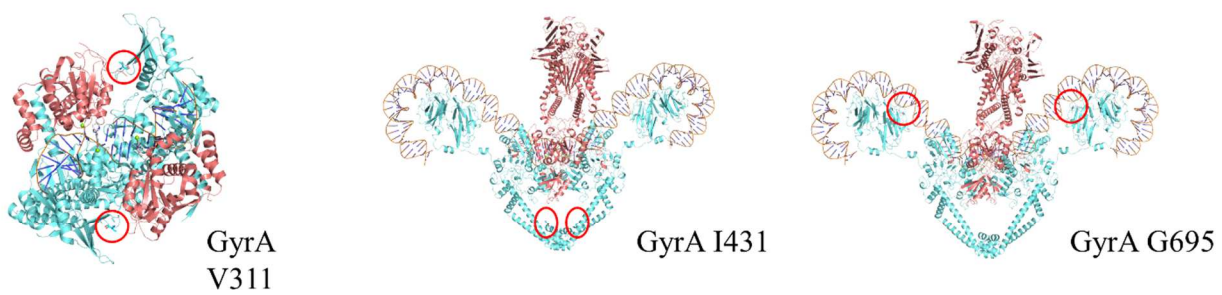
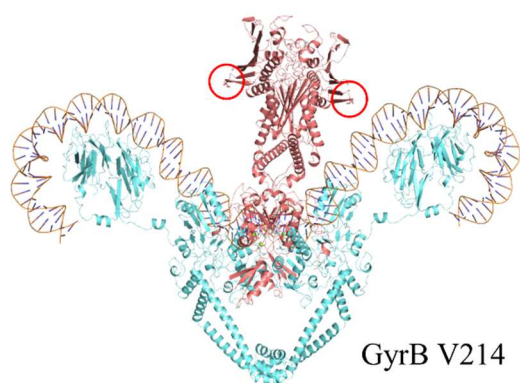
sp | P0AES6 | GYRB\_ECOLI | DHEFITGGEYRRIC TLGEKLRGLLEEDAFIERGERRQPVASFEQALDWLVKESRRGLSIQ | 737  
SP | P9GW45 | GYRB\_MYCTU | -----KKINKEDGIQ | 608  
sp | Q59533 | GYRB\_MYCLE | -----KKINKEDGIQ | 611  
: \* : : : : : \* :

sp | P0AES6 | GYRB\_ECOLI | RYKGLGEMNPEQLWETTMDPESRRMLRVTVKDAIAADQLFTTLMGDAVEPRRAFIEENAL | 797  
SP | P9GW45 | GYRB\_MYCTU | RYKGLGEMDAKELWETTMDPSVRVLRQVTLDDAAAAD E L F S I L M G E D V D A R R S F I T R N A K | 668  
sp | Q59533 | GYRB\_MYCLE | RYKGLGEMDAKELWETTMDP SVRVLRQVTLDDAAAAD E L F S I L M G E D V D A R R S F I T R N A K | 671  
\*\*\*\*\* : : : \* \* \* \* \* \* \* : \* : : : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* : \* \* :

sp | P0AES6 | GYRB\_ECOLI  
SP | P9GW45 | GYRB\_MYCTU  
sp | Q59533 | GYRB\_MYCLE

KAANIDI 804  
DVRFLDV 675  
DVRFLDV 678  
: : : : \* :

**Figure 3.** *M. leprae* DNA gyrase model. (A) Model of the full-length structure of *M. leprae* gyrase in complex with DNA and moxifloxacin. Protein is shown in cartoon representation with transparent surfaces, with GyrA in blue and GyrB in pink. DNA is shown in orange. Moxifloxacin is shown in grey in stick representation. A zoom on the QRDR region is shown to localize residues P87, G89, A91 and S92 in GyrA and D464 and T503 in GyrB. The third panel is an extended zoom of this QRDR region to localize residue R107 in GyrA. The substituted amino acids are shown in stick representation. (B) Residues V311, I431 and G695 found mutated in GyrA. Top view of the *M. leprae* DNA gyrase model in the first panel to localize V311. The same representation, as in panel A, was used for the protein. (C) Residue V214 in GyrB. The same representation, as in panel A, was used for the protein.

**A****B****C**

**Table 1.** Comparison of the GyrA numbering system and the corresponding regions of *E. coli* and *M. tuberculosis*, and the proposed consensus numbering system for *M. leprae* GyrA with and without the intein.

Substitution observed*	<i>E. coli</i> <sup>a</sup>	<i>M. tuberculosis</i> <sup>b</sup>	<i>M. leprae</i> <sup>c</sup> without intein	<i>M. leprae</i> GyrA_intein
Pro→Leu	79	87	87	NA <sup>d</sup>
Gly→Cys	81	88	89	NA <sup>d</sup>
Gly→Ala	81	88	89	NA <sup>d</sup>
Ala→Val	82	90	91	NA <sup>d</sup>
Ala→Thr	82	90	91	NA <sup>d</sup>
Ala→Pro	82	90	91	NA <sup>d</sup>
Ser→Ala	83	91	92	NA <sup>d</sup>
Asp→Gly	87	94	95	NA <sup>d</sup>
Asp→Asn	87	94	95	NA <sup>d</sup>
Leu→Pro	89	98	97	NA <sup>d</sup>
Arg→Leu	99	106	107 <sup>f</sup>	NA <sup>d</sup>
Ser→Leu	NA <sup>e</sup>	NA <sup>e</sup>	NA <sup>e</sup>	177
Gly→Glu	NA <sup>e</sup>	NA <sup>e</sup>	NA <sup>e</sup>	232
Val→Ile	299	310	311	NA <sup>d</sup>
Gly→Asp <sup>g</sup>	-	-	362 <sup>g</sup>	NA <sup>d</sup>
Ile→Thr	453	430	431	NA <sup>d</sup>
Gly→Arg	715	694	695	NA <sup>d</sup>

The Amino acids inside the QRDR of the GyrA subunit are enclosed in the bold box and range from 67 to 106 for *E. coli*, 74 to 113 for *M. tuberculosis*, and 75 to 114 for *M. leprae*; the amino acids of the GyrA intein of *M. leprae* range from 1 to 420.

\*substitution observed in *M. tuberculosis* and/or *M. leprae* clinical strains

<sup>a</sup> P9WG47

<sup>b</sup> P9WG47

<sup>c</sup> Q57532

<sup>d</sup> not applicable because mutations are located outside the intein

<sup>e</sup> not applicable because mutations are located inside the intein

<sup>f</sup> this study reported an A107L substitution, but when we looked at the sequence of the QRDR of GyrA, we did not find an alanine at this position but an arginine. After consulting with the authors, A107L was found to be erroneous. Here we give R107L, the correct substitution.

<sup>g</sup> this study initially reported a G362D substitution, but when we looked at the sequence of GyrA, we did not find a glycine at this position but a methionine. Unfortunately, we were not able to obtain clarification from the authors. Therefore, we cannot propose a corresponding number in the *E. coli* and *M. tuberculosis* numbering systems.[40]

**Table 2.** Comparison of the four GyrB numbering systems for *M. tuberculosis* described in the literature, the corresponding region in *E. coli* and the proposed consensus numbering system for *M. leprae*.

Substitution observed*	<i>E. coli</i> <sup>a</sup>	<i>M. tuberculosis</i> GyrB sequence (year of publication)				<i>M. leprae</i> <sup>f</sup>
		1994 <sup>b</sup>	1998 <sup>c</sup>	2000 <sup>d</sup>	2002 <sup>e</sup>	
Val→Gly	210	ND	252	224	213	<b>214</b>
Arg→Cys	411	480	485	457	446	<b>449</b>
Ser→Phe	412	481	486	458	447	<b>450</b>
Asp→Ala	426	495	500	472	461	<b>464</b>
Asp→Asn	426	495	500	472	461	<b>464</b>
Asp→His	426	495	500	472	461	<b>464</b>
Gly→Ala	435	504	509	481	470	<b>473</b>
Asp→Ala	459	528	533	505	494	<b>497</b>
Asn→Asp	464	533	538	510	499	<b>502</b>
Asn→Lys	464	533	538	510	499	<b>502</b>
Asn→Thr	464	533	538	510	499	<b>502</b>
Thr→Asn	465	534	539	511	500	<b>503</b>
Thr→Ile	465	534	539	511	500	<b>503</b>
Thr→Pro	465	534	539	511	500	<b>503</b>
Glu→Asp	466	535	540	512	501	<b>504</b>
Glu→Val	466	535	540	512	501	<b>504</b>
Ala→Thr	469	538	543	515	504	<b>507</b>
Ala→Val	469	538	543	515	504	<b>507</b>
Asp→Tyr	482	552	557	529	518	<b>521</b>
Gln→His	503	572	577	549	538	<b>541</b>

The QRDR of GyrB in accordance with that of *E. coli* is in the bold box with a hatched line at the bottom, whereas the QRRD of GyrB in accordance with that of *M. tuberculosis* [28], ranging from 426 to 466 for *E. coli*, 461 to 501 for *M. tuberculosis* in the proposed numbering system and 464 to 504 for *M. leprae*, is in the solid bold box.

\*substitution observed in *M. tuberculosis* or *M. leprae* clinical strains.

<sup>a</sup> P0AES6

<sup>b</sup> AAA83016.1. [49] is an obsolete entry and has been replaced in the alignment by P9GW45

<sup>c</sup> CABO2426.1. [50] is an obsolete entry and has been replaced in the alignment by P9GW45

<sup>d</sup> Zhou et al. [51]

<sup>e</sup> P0C5C5|1-675, [44] in italic since it is the numbering system that should be used for *M. tuberculosis*. [25]

<sup>f</sup> Q59533 replaces the obsolete entry *M. leprae* Cosmid B1770 Z70722 (used by Matrat *et al*)[16]



**Table 3.** Clinical *M. leprae* isolates studied: fluoroquinolone susceptibility and molecular detection methods used in each primary study included in the review.

Type of collection	Type of leprosy	Nb of cases studied <sup>a</sup>	Molecular detection method	DNA region studied	Nb of cases with DNA gyrase substitution	Nb of relapse cases	Mouse footpad DST	Reference
Colombian cases	MB	941	PCR seq	QRDR_A	11	560	no	[32]
Indian cases	203 MB/ 47 PB	250	PCR seq	QRDR_A	8	239	no	[52]
Cases reported to French National Reference Center	MB	160	PCR seq and DNA strip	QRDR_A	2	33	no	[34]
Indian cases	ND	111	PCR seq	QRDR_A	10	111	no	[38]
Japanese, Indonesian, Pakistani and Philippine cases	ND	88	PCR seq	QRDR_A	5	ND	no	[21]
Chinese cases	72 MB/9 PB	81	PCR seq	QRDR_A	21	8	no	[31]
Brazilian cases	ND	79	PCR seq	QRDR_A	6	ND	no	[53]
Brazilian cases	59MB/18PB	77	PCR seq	QRDR_A	2	77	no	[35]
Indian and Nepalese cases	53 BT/8 TT/8 BL/3 BB/ 4 healed/fibrosed 1 axonopathy <sup>b</sup>	77	PCR seq	QRDR_A	6	0	no	[29]
Chinese cases	ND	61	PCR seq	QRDR_A	1	ND	no	[37]
Brazilian cases	ND	45	PCR seq	QRDR_A	1	ND	no	[54]
Indian cases	MB	38	PCR seq	QRDR_A	8	3	no	[33]
Mexican cases	36MB/2PB	38	PCR seq	QRDR_A	1	30	no	[55]
Brazilian cases	18MB/ 10PB	28	PCR seq	QRDR_A	2	1	no	[56]
Cases reported to the French National Reference Center	MB	10	PCR seq	QRDR_A	1	NA	yes	[15]
Case report from Mali	MB	1	PCR seq	QRDR_A	1	1	yes	[13]
Case report from Japan	MB	1	PCR seq	QRDR_A	1	1	yes	[22]

Case report from Japan	MB	1	PCR seq	QRDR_A	1	1	yes	[23]
Case report from India	PB	1	PCR seq	QRDR_A	1	1	no	[30]
Case report from Madagascar	MB	1	DNA strip test	QRDR_A	1	1	no	[36]
Colombian cases	MB	200	PCR seq	QRDR_AB	1	34	no	[39]
Chinese cases	MB	290	PCR seq	QRDR_AB	8	2	no	[40]
Korean cases	MB	104	PCR seq	QRDR_AB	2	ND	no	[3]
Korean cases	MB	7	PCR seq	QRDR_AB	1	7	no	[41]
Worldwide cases	ND	154	WGS	WGS	13	ND	no	[26]
<b>Total number of isolates</b>	<b>1985 MB/76PB/2134 MB/ 94PB/ 53 BT/ 8TT/ 8BL/ 3BB/ 4 healed fibrosed and 1 axonopathy</b>	<b>2884</b>			<b>115</b>	<b>1110</b>	<b>3/24</b>	

DST: Drug Susceptibility Testing; NA: not applicable; ND: no data; PCR seq: PCR and DNA sequencing; WGS: whole genome sequencing;

DNA strip test: GenoType LeptraeDR<sup>®</sup> (Hain, Lifescience); QRDR\_A: QRDR gyrase A; QRDR\_AB: QRDR gyrase A and B.

<sup>a</sup> number of strains with their gyrase genes sequenced (*gyrA*, *gyrB* or *gyrA* and *gyrB*).

<sup>b</sup> BT: borderline tuberculoid; TT: tuberculoid; BL: borderline lepromatous; BB: mid borderline.

**Table 4.** GyrA and GyrB substitutions reported in *M. leprae* clinical cases.

<b>Subunit</b>	<b>Substitution named by the author</b>	<b>Substitution in the proposed numbering system</b>	<b>Nb of isolates with this mutation</b>	<b>Prior FQ use</b>	<b>Relapse</b>	<b>Reference</b>
<b>Single substitution in GyrA</b>						
GyrA	P89L	P87L	1	ND	ND	[53]
GyrA	<b>G89C</b>	<b>G89C</b>	2 <sup>a</sup>	ND	yes	[32]
GyrA	<b>G89C</b>	<b>G89C</b>	1	ND	ND	[21]
GyrA	<b>G89C</b>	<b>G89C</b>	1	ND	1	[30]
GyrA	<b>G89C</b>	<b>G89C</b>	1	no	no	[29]
GyrA	<b>G89C</b>	<b>G89C</b>	1	no	no	[30]
GyrA	<b>G89A</b>	<b>G89A</b>	1	no	no	[29]
GyrA	<b>A91V</b>	<b>A91V</b>	21	yes for 20	yes for 1	[31]
GyrA	<b>A91V</b>	<b>A91V</b>	9	yes for 6	yes for 4	[32]
GyrA	<b>A91V</b>	<b>A91V</b>	8	yes for 3	yes for 5	[33]
GyrA	<b>A91V</b>	<b>A91V</b>	7	ND	yes	[52]
GyrA	<b>A91V</b>	<b>A91V</b>	4	yes for 2	yes for 2	[21]
GyrA	<b>A91V</b>	<b>A91V</b>	4	ND	yes for 2	[40]
GyrA	<b>A91V</b>	<b>A91V</b>	2	yes for 1	yes for 1	[26]
GyrA	<b>A91V</b>	<b>A91V</b>	2	yes	no	[34]
GyrA	<b>A91V</b>	<b>A91V</b>	2	yes for 1	yes	[35]
GyrA	<b>A91V</b>	<b>A91V</b>	1	ND	yes	[3]
GyrA	<b>A91V</b>	<b>A91V</b>	1	yes	yes	[13]
GyrA	<b>A91V</b>	<b>A91V</b>	1	yes	yes	[22]
GyrA	<b>A91V</b>	<b>A91V</b>	1	no	yes	[23]
GyrA	<b>A91V</b>	<b>A91V</b>	1	yes	no	[36]
GyrA	<b>A91V</b>	<b>A91V</b>	1	yes	no	[37]
GyrA	<b>A91V</b>	<b>A91V</b>	1	ND	ND	[54]
GyrA	<b>A91V</b>	<b>A91V</b>	1	ND	yes	[38]
GyrA	<b>A91V</b>	<b>A91V</b>	1	ND	yes	[55]
GyrA	<b>A91V</b>	<b>A91V</b>	1	no	no	[29]

GyrA	<b>A91V</b>	<b>A91V</b>	1	ND	yes	[53]
GyrA	<b>A91P</b>	<b>A91P</b>	2	no	no	[29]
GyrA	A91T	A91T	3 <sup>b</sup>	not for leprosy	yes	[38]
GyrA	<b>S92A</b>	<b>S92A</b>	6 <sup>c</sup>	not for leprosy	yes	[38]
GyrA	<b>S92A</b>	<b>S92A</b>	1	ND	yes	[52]
GyrA	L97P	L97P	2	ND	yes for 1	[56]
GyrA	R107L <sup>d</sup>	R107L	1	under treatment	no	[39]
GyrA	V73II	V311I	1	ND	ND	[26]
GyrA	G362D	<sup>e</sup>	4	ND	1	[40]
GyrA	I851T	I431T	1	ND	ND	[26]
GyrA	G1115R	G695R	2	ND	ND	[26]
GyrA	S307L	S177L GyrA_intein	2	ND	ND	[26]
GyrA	G362E	G232E GyrA_intein	1	ND	ND	[26]
<b>Multiple substitutions in GyrA</b>						
GyrA	<b>G89C + A91V</b>	<b>G89C + A91V</b>	2	ND	yes	[32]
GyrA	A91T + <b>S92A</b>	A91T + <b>S92A</b>	1	no	yes	[38]
<b>Single substitution in GyrB</b>						
GyrB	V214G <sup>f</sup>	V214G	1	ND	ND	[26]
GyrB	V214G	V214G	1	no	ND	[40]
GyrB	D205N	<b>D464N</b>	1	ND	yes	[3]
GyrB	D205N	<b>D464N</b>	1	yes	yes	[41]
GyrB	<b>D464N<sup>g</sup></b>	<b>D464N</b>	1	ND	ND	[26]
GyrB	T503I <sup>h</sup>	T503I	1	ND	ND	[26]
<b>Multiple substitutions in GyrA and GyrB</b>						
GyrA-B	<b>A91V (A) + D464N (B)</b>	<b>A91V (A) + D464N (B)</b>	1	ND	ND	[26]
GyrA-B	V73II (A) + T503I (B)	V311I (A) + T503I (B)	1	ND	ND	[26]

GyrA-B	I851T (A) + V214G (B)	I431T(A) + V214G (B)	1	ND	ND	[26]
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ND: no data

Substitutions demonstrated to confer fluoroquinolone resistance in *M. leprae* are in bold; substitutions not in bold have not been assessed for conferring resistance.

<sup>a</sup> associated with GyrA A91V substitution.

<sup>b</sup> 1 associated with GyrA S92A substitution.

<sup>c</sup> 1 associated with GyrA A91T substitution.

<sup>d</sup> this study reported an A107L substitution, but when we looked at the sequence of the QRDR of GyrA, we did not find an alanine at this position but an arginine. After consulting with the authors, A107L was found to be erroneous. Here we give R107L, the correct substitution. [39]

<sup>e</sup> this study initially reported a G362D substitution, but when we looked at the sequence of GyrA, we did not find a glycine at this position but a methionine. Unfortunately, we were not able to obtain clarification from the authors. Therefore, we cannot propose a corresponding number in the *E. coli* and *M. tuberculosis* numbering systems.

<sup>f</sup> associated with GyrA I431T substitution.

<sup>g</sup> associated with GyrA A91V substitution.

<sup>h</sup> associated with GyrA V731I substitution.

**Table 5.** Effect of substitutions in GyrA and GyrB identified in *M. leprae* subsequent to inhibition of gyrase activity by fluoroquinolone: correlation with *M. leprae* resistance to fluoroquinolone.

Gyrase subunit alteration		IC <sub>50</sub> (mg/L)		Study reference
GyrA	GyrB	OFX	MXF	
WT	WT	10	2	[46]
WT	WT	15	6	[16]
WT	WT	6.8+/-0.8	1.5+/-0.3	[57]
WT	WT	5.7+/-0.8	1.7+/-0.3	[48]
WT	WT	nd	1.1+/-0	[58]
<b>G89C</b>	WT	160	30	[16]
<b>G89C</b>	WT	nd	22.6+/-2.9	[58]
<i>A91V<sup>a</sup></i>	WT	80	25	[16]
<i>A91V<sup>a</sup></i>	WT	nd	2.1+/-0.1	[58]
<b>D95G<sup>b</sup></b>	WT	161.2+/-44.2	21.5+/-4.7	[57]
<b>D95G<sup>b</sup></b>	WT	nd	12.2+/-1.3	[58]
<b>D95N<sup>b</sup></b>	WT	262.3+/-105.8	34.7+/-3.1	[57]
WT	<i>D205N</i>	20	6	[16]
WT	<b>D464N</b>	53.9+/-9	4.1+/-0.4	[48]
WT	<b>N502D<sup>b</sup></b>	106.6+/-25.1	17.8+/-2.6	[48]
WT	<b>E504V<sup>b</sup></b>	34.6+/-4.3	13.9+/-0.6	[48]

IC<sub>50</sub>, 50% inhibitory concentration (measured by inhibition of 50% of DNA supercoiling);

MXF, moxifloxacin; OFX, Ofloxacin; WT wild type.

Substitutions demonstrated not to confer fluoroquinolone resistance in *M. leprae* are in italics.

Substitutions demonstrated to confer fluoroquinolone resistance in *M. leprae* are in bold.

<sup>a</sup> the impact of this substitution was also demonstrated using the mouse footpad technique.

[7,13,15,21–23]

<sup>b</sup> substitution never described in a clinical isolate of *M. leprae*.