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ABSTRACT

Background

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

Objectives

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

Data sources

Data source was PubMed.

Study eligibility criteria

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

Results

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

Conclusion

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

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

INTRODUCTION

Mycobacterium leprae, the etiological agent of leprosy, was responsible for 193 840 new cases in 2019. [1] Additionally, in 2019, 3897 cases of leprosy relapses were reported by 55 countries, representing 2% of the total case notification. [1] Relapses can be due to non-adherence to the recommended multidrug therapy (MDT) or to antimicrobial resistance (AMR). [2–5]

Monitoring AMR remains challenging because *M. leprae* does not grow *in vitro*. Two methods can be used for AMR monitoring: an *in vivo* phenotypic method using Shepard's mouse footpad model [6] which requires a high level of expertise and is expensive and time-consuming (ca. 12 months); or genotypic methods, such as PCR sequencing, a line probe assay (i.e. the DNA STRIP technology GenoType LepraeDR® Hain Lifescience) [7] or whole-genome sequencing (WGS). [8] Because of the complexity of the phenotypic method, genotypic methods are currently the main methods used to diagnose AMR in *M. leprae*. Reliable data concerning mutations and their impact on AMR are required, especially since the presence of a mutation in a gene encoding a drug target or an activator does not necessarily confer resistance. [9]

In this review, we focused on the fluoroquinolones (FQ) since (i) they are effective and powerful bactericidal drugs against *M. leprae* [10–12] and (ii) their use for the treatment of other infections has promoted the emergence of resistance, as in the case of *M. tuberculosis*. [13,14] According to the first global resistance data published in 2018, with resistance to FQ diagnosed using genotypic methods,1,33% of 1581 *M. leprae* isolates studied were resistant to ofloxacin. [5] FQ targets are generally the type II topoisomerases (i.e. DNA gyrase and topoisomerase IV), but with *M. leprae* lacking topoisomerase IV, the DNA gyrase is the sole target of FQ in this organism. [8]

The purpose of our review was (i) to characterize all DNA gyrase gene mutations described in clinical strains of *M. leprae* and (ii) to distinguish between those associated with FQ resistance and those associated with susceptibility. The existing tool for this latter purpose was a model

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

METHODS

Definitions

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

Biochemical method is an *in vitro* technique enabling to evaluate the impact of DNA gyrase mutations on FQ efficacy by measuring the FQ concentrations required to inhibit the DNA supercoiling activity of the DNA gyrase (IC_{50}) (Table 5). Comparing the FQ concentrations needed for the WT *M. leprae* DNA gyrase and the mutated enzymes enables to evaluate the impact of mutations of DNA gyrase on susceptibility to FQ. These data correlate with *in vivo* efficacy of FQ. [16]

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

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

Research methodology

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

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

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

Data acquisition

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

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

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

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

Modeling

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

Quality control

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

RESULTS

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

GyrA

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

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

GyrB

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

Findings

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

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

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

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

Mutations in *gyrA* (Tables 1, 4)

Among the 18 substitutions described in GyrA, nine were inside and seven outside the QRDR, and two were inside the intein (Table 1). The A91 substitution was the most prevalent. It was found in 75 (68%) of the 110 clinical strains that harbored a substitution in GyrA. Three different substitutions were reported at this position, A91V, A91T and A91P. The A91V substitution was the most prevalent (70, 3 and 2 strains harbored A91V, A91T and A91P substitutions, respectively). Interestingly, for the three strains carrying the A91V substitution that were tested, the phenotypic mouse footpad method confirmed the diagnosis of resistance 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

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

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

DISCUSSION

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

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

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

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

With the emergence of the WGS techniques, mutations outside the QRDR are more often found, increasing the need for a unique numbering system for both GyrA and GyrB. For substitutions in GyrA, we propose a numbering system based on the alignment of *M. leprae* for GyrA QRDR between codons 75 and 114, not taking into account the intein, and a numbering system specific 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

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

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

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

Currently, analyzing FQ resistance exclusively by sequencing of the QRDR of *gyrA* may lead to a possible underestimation of FQ resistance in leprosy. While WGS appears to be the most 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

Transparency declaration

All authors declare no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; and no other relationships or activities that could appear to have influenced the submitted work.

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Author contributions

AA designed the research. AC, FM, FR, SP, CM, EC and AA conducted the research. AC wrote the first draft of the paper. FM, FR, SP, CM, EC and AA contributed to the writing of the paper. All authors contributed to the data interpretation, revised each draft for important intellectual content, and read and approved the final manuscript.

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

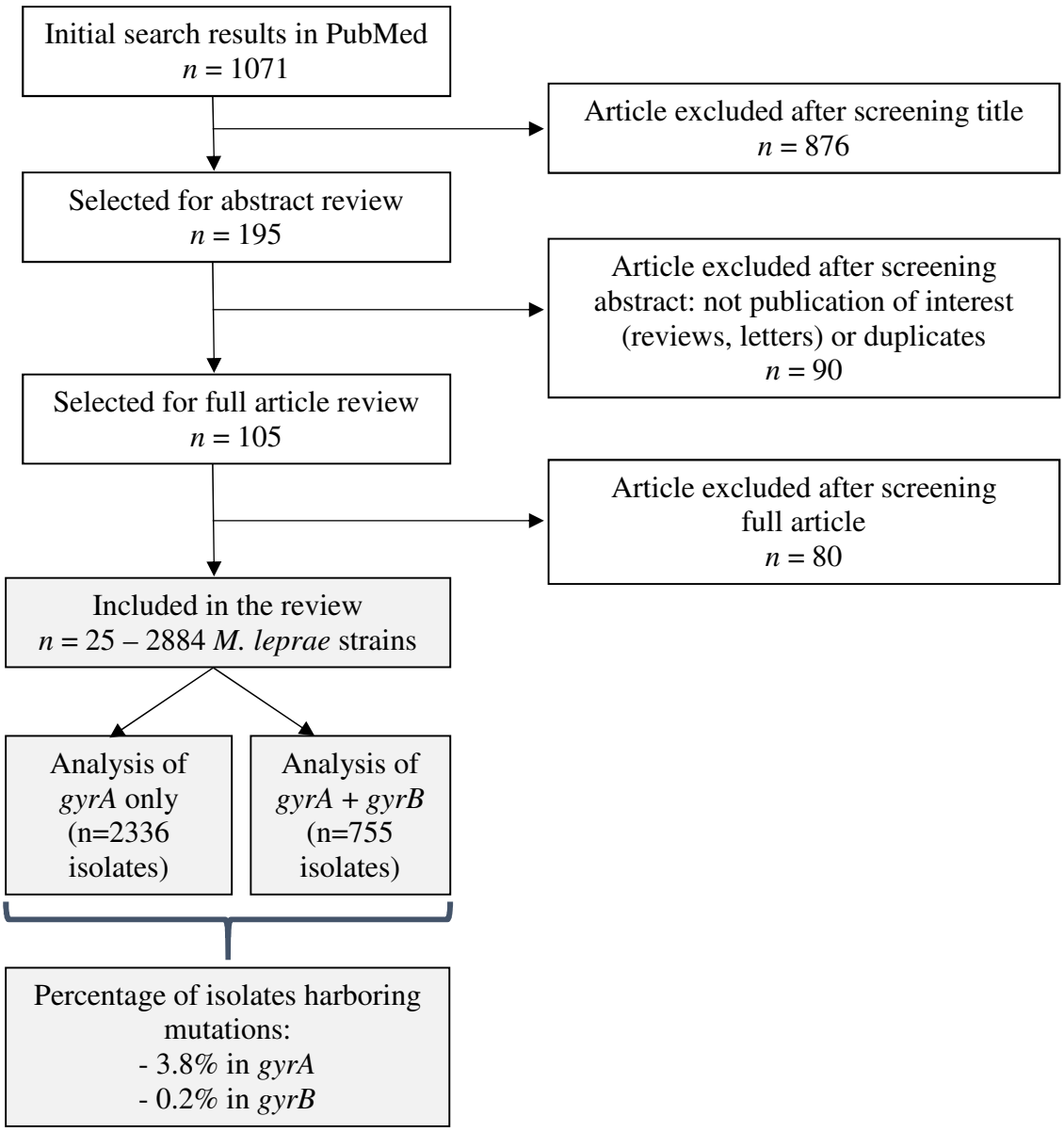
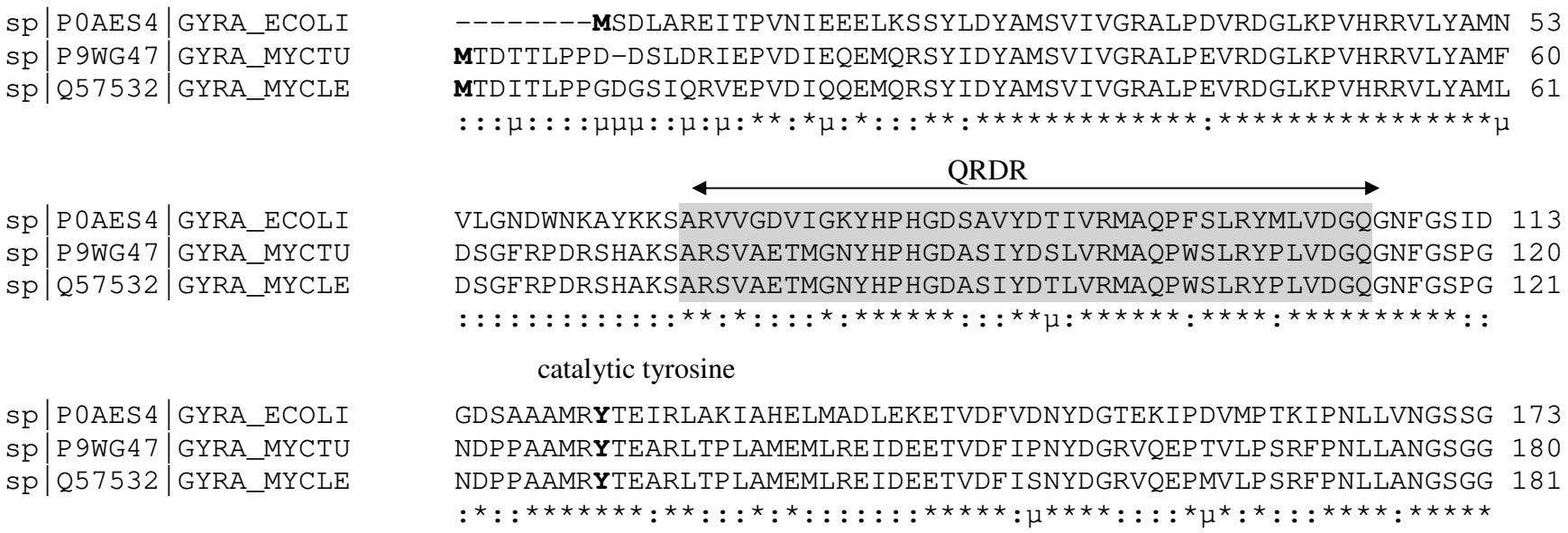


Figure 1. Sequence alignment of the GyrA subunits of *M. tuberculosis* (P9WG47), *E. coli* (P0AES4), and *M. leprae* (Q57532), and proposed numbering system for the *M. leprae* GyrA subunit.

* = identical residue between *E. coli*, *M. tuberculosis* and *M. leprae*, : = identical residue between *M. tuberculosis* and *M. leprae* but different from *E. coli* and µ = non-conserved substitution between *M. tuberculosis* and *M. leprae*. The QRDR is highlighted in grey. The start codons, the GyrA-box and GyrA-box-like motifs are in bold. The tyrosine in the catalytic region is indicated.



```
sp|P0AES4|GYRA_ECOLI        IAVGMATNIPPHNLTEVINGCLAYID----DEDISIEGLMEHIPGPDFPTAAIINGRRGI 229
sp|P9WG47|GYRA_MYCTU        IAVGMATNIPPHNLRELADAVFWALENHDADEEETLAAVMGRVKGPDFPTAGLIVGSQGT 240
sp|Q57532|GYRA_MYCLE        IAVGMATNIPPHNLYELADAVFWCLENHDADEETMLVAVMERVKGPDFPTAGLIVGSQGI 241
                            **************µ*:::::::µ:::::::**:µµ:µ::*µ:::********::*:*::*µ

sp|P0AES4|GYRA_ECOLI        EEAYRTGRGKVYIRARAEVEVDAKTGRETIIVHEIPYQVNKARLIEKIAELVKEKRVEGI 289
Sp|P9WG47|GYRA_MYCTU        ADAYKTGRGSIRMRGVVEVEEDSR-GRTSLVITELPYQVNHDNFITSIAEQVRDGKLAGI 299
Sp|Q57532|GYRA_MYCLE        ADAYKTGRGSIRIRGVVEVEEDSR-GRTSLVITELPYQVNHDNFITSIAEQVRTGRLAGI 300
                            ::**:*****:::µ*:::***:*:::**:::::::*:*****::::*::***:*:µ:µ::**

sp|P0AES4|GYRA_ECOLI        SALRDES-DKDGMRIVIEVKRDAVGEVVLNNLYSQTQLQVSFGINMVALHHGQPKIMNLK 348
sp|P9WG47|GYRA_MYCTU        SNIEDQSSDRVGLRIVIEIKRDAVAKVVINNLYKHTQLQTSFGANMLAIVDGVPRTLRLD 359
sp|Q57532|GYRA_MYCLE        SNVEDQGSDRVGVRIVIEIKRDAVAKVVLNNLYKHTQLQTSFGANMLSIVDGVPRTLRLD 360
                            *:µ:*:µ:*::*µ***********:**µ****::****:***:**:µ:::*:*::::*:

sp|P0AES4|GYRA_ECOLI        DIIAAFVRHRREVVTRRTIFELRKARDRAHILEALAVALANIDPIIELIRHAPTPAEAKT 408
sp|P9WG47|GYRA_MYCTU        QLIRYYVDHQLDVIVRRTTYRLRKANERAHILRGLVKALDALDEVIALIRASETVDIARA 419
sp|Q57532|GYRA_MYCLE        QMICYYVEHQLDVIVRRTTYRLRKANERAHILRGLVKALDALDEVITLIRASQTVDIARV 420
                            :µ*µ::*µ*:::*::***::****::*****::*::**:::*::*µ***::µ*:::*:µ

sp|P0AES4|GYRA_ECOLI        ALVANPWQLGNVAAMLERAGDDAARPEWLEPEFGVRDGLYYLTEQQAQAILDLRLQKLTG 468
sp|P9WG47|GYRA_MYCTU        GLIE--------------------------------LLDIDEIQAQAILDMQLRRLAA 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        LERQRIIDDLAKIEVEIADLGDILAKPERRRGIIRNELTEIAEKYGDDRRTRIIAVDGDV 506
                            **:::::*::::::µ:**:*µ:**::::*µ:::µ*µ**µ::µµµµ**:***:*:*µ::*:
```

GyrA-box

```
sp|P0AES4|GYRA_ECOLI        NLEDLITQEDVVVTLSHQGYVKYQPLSEYEAQRRGGKGKSAARIKEEDFIDRLLVANTHD 588
sp|P9WG47|GYRA_MYCTU        SDEDLIAREDVVVTITETGYAKRTKTDLYRSQKRGGKGVQGAGLKQDDIVAHFFVCSTHD 565
sp|Q57532|GYRA_MYCLE        NDEDLIAREEVVVTITETGYAKRTKTDLYRSQKRGGKGVQGAGLKQDDIVRHFFVCSTHD 566
                            µ:*****::*µ**********:*:*******::*:*******::*::*::*::µ:::*::***

sp|P0AES4|GYRA_ECOLI        HILCFSSRGRVYSMKVYQLPEATRGARGRPIVNLLPLEQDERITAILPVTEFEEGVKVFM 648
sp|P9WG47|GYRA_MYCTU        LILFFTTQGRVYRAKAYDLPEASRTARGQHVANLLAFQPEERIAQVIQIRGYTDAPYLVL 625
sp|Q57532|GYRA_MYCLE        WILFFTTQGRVYRAKAYELPEASRTARGQHVANLLAFQPEERIAQVIQIRSYEDAPYLVL 626
                            µ**:*:::****:::::µ****:*:***:::::***:::::::::::µ:µ:::::::

sp|P0AES4|GYRA_ECOLI        ATANGTVKKTVLTEFNRLRTAGKVAIKLVDGDELIGVDLTSGEDEVMLFSAEGKVVRFKE 708
sp|P9WG47|GYRA_MYCTU        ATRNGLVKKSKLTDFDSNRSGGIVAVNLRDNDELVGAVLCSAGDDLLLVSANGQSIRFSA 685
sp|Q57532|GYRA_MYCLE        ATRAGLVKKSKLTDFDSNRSGGIVAINLRDNDELVGAVLCAADGDLLLVSANGQSIRFSA 686
                            **:µ*:***::**:*:::::::::**µ:*:*:***:*::*:µ:µµ:::*:**:*:::**::
                                                                                GyrA-box-like

sp|P0AES4|GYRA_ECOLI        S--SVRAMGCNTTGVRGIRLGEGDKVVSLIVPRGDGAILTATQNGYGKRTAVAEYPTKSR 766
sp|P9WG47|GYRA_MYCTU        TDEALRPMGRATSGVQGMRFNIDDRLVSLNVVREGTYLLVATSGGYAKRTAIEEYPVQGR 745
sp|Q57532|GYRA_MYCLE        TDEALRPMGRATSGVQGMRFNADDRLLSLNVVREDTYLLVATSGGYAKRTSIEEYPMQGR 746
                            :::::*:**::*:**:*:*::µ::::::**:*:*:µ:::*:**::::*:***µ::***µ::*

sp|P0AES4|GYRA_ECOLI        ATKGVISIKVTERNGLVVGAVQVDDCDQIMMITDAGTLVRTRVSEISIVGRNTQGVILIR 826
sp|P9WG47|GYRA_MYCTU        GGKGVLTVMYDRRRGRLVGALIVDDDSELYAVTSGGGVIRTAARQVRKAGRQTKGVRLMN 805
sp|Q57532|GYRA_MYCLE        GGKGVLTVMYDRRRGSLVGAIVVDEDSELYAITSGGGVIRTTARQVRQAGRQTKGVRLMN 806
                            ::***:::::::*:*µ:***µµ**µ:::::::*::*::::**µ:::::µ:**:*:**:*::

sp|P0AES4|GYRA_ECOLI        TAEDENVVGLQRVAEPVDEEDLDTIDGSAAEGDDEIAPEVDVDDEPEEE 875
sp|P9WG47|GYRA_MYCTU        LGEGDTLLAIARNAEESGDDNAVDANGADQTGN---------------- 838
sp|Q57532|GYRA_MYCLE        LGEGDTLLAIARNAEESADGVSVKVMISRSRVLSFFGSDSNTSPDRT-- 853
                            ::*:::::::::*:**::µ:µµµµµµµµµµµµµµµµµµµµµµµµµµµµµµµµµµµµµµµµ
```

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 µ = 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        -------MSNSYDSSSIKVLKGLDAVRKRPGMYIGDTDDGTGLHHMVFEVVDNAIDEALA 53
sp|P9GW45|GYRB_MYCTU        MAAQKKKAQDEYGAASITILEGLEAVRKRPGMYIGSTGE-RGLHHLIWEVVDNAVDEAMA 59
sp|Q59533|GYRB_MYCLE        MAAQR-KAQDEYGAASITILEGLEAVRKRPGMYVGSTGE-RGLHHLIWEVVDNSVDEAMA 58
                            µ::::µ:::::*:::**:::::**:*********µ*:*::::*****::*****µ:***:*

sp|P0AES6|GYRB_ECOLI        GHCKEIIVTIHADNSVSVQDDGRGIPTGIHPEEGVSAAEVIMTVLHAGGKFDD--NSYKV 111
sp|P9GW45|GYRB_MYCTU        GYATTVNVVLLEDGGVEVADDGRGIPVATHAS-GIPTVDVVMTQLHAGGKFDS--DAYAI 116
sp|Q59533|GYRB_MYCLE        GYATQVDVRLFDDGSVEVADNGRGIPVAVHAT-GVPTVDVVMTQLHAGGKFGGKDSGYNV 117
                            *:::µ:µ*µ:µµ*:µ*:*:*µ*****::µ*:µ:*µ::::*:**:*******µµµµµµ*µµ

sp|P0AES6|GYRB_ECOLI        SGGLHGVGVSVVNALSQKLELVIQREGKIHRQIYEHGVPQAPLAVTGETEKTGTMVRFWP 171
sp|P9GW45|GYRB_MYCTU        SGGLHGVGVSVVNALSTRLEVEIKRDGYEWSQVYEKSEPLG-LKQGAPTKKTGSTVRFWA 175
sp|Q59533|GYRB_MYCLE        SGGLHGVGVSVVNALSTRVEVDIKRDGYEWSQFYDKAVPGI-LKQGEATEATGTTIRFWA 176
                            ****************::µ*:µ*:*:*::::*µ*µ:µµ*µµ:*::::µ*µµ**µ:µ***:

sp|P0AES6|GYRB_ECOLI        SLETFTNVTEFEYEILAKRLRELSFLNSGVSIRLRDKRDGKED----------------- 214
sp|P9GW45|GYRB_MYCTU        DPAVFE-TTEYDFETVARRLQEMAFLNKGLTINLTDERVTQDEVVDEVVSDVAEAP--KS 232
sp|Q59533|GYRB_MYCLE        DPDIFE-TTKYDFGTVARRIQEVAFLNKGLTINLVDERVKQDEVVDDVVSDTAEAPVAMT 235
                            ::µµ*:::*µ:::µ::*:*µ:*µ:***:*::*:*µ*:*:µ::::::µ::::µ::::µµµµ
```

```
sp|P0AES6|GYRB_ECOLI          ----------------HFHYEGGIKAFVEYLNKNKTPIHPNIFYFSTEKDGIGVEVALQ 257
sp|P9GW45|GYRB_MYCTU          ASERAAESTAPHKVKSRTFHYPGGLVDFVKHINRTKNAIHSSIVDFSGKGTGHEVEIAMQ 292
sp|Q59533|GYRB_MYCLE          VEEKSTESSAPHKVRHRTFHYPGGLVDFVKHINRTKTPIQQSIIDFDGKGAGHEVEVAMQ 295
                              µµ:µµµ::µ:::::µµ::***:**:::**:::*::*µµ*µµ:*µ:*µ:::µ*::**µ*:*

sp|P0AES6|GYRB_ECOLI          WNDGFQENIYCFTNNIPQRDGGTHLAGFRAAMTRTLNAYMDKEGYSKKAKVSATGDDARE 317
sp|P9GW45|GYRB_MYCTU          WNAGYSESVHTFANTINTHEGGTHEEGFRSALTSVVNKYAKDRKLLKDKDPNLTGDDIRE 352
sp|Q59533|GYRB_MYCLE          WNGGYSESVHTFANTINTHEGGTHEEGFRSALTSVVNKYAKDKKLLKDKDPNLTGDDIRE 355
                              **µ*::*:::::*:*:*:::::****::***:*:*:::*:*:::µ:::*::::::****:**

sp|P0AES6|GYRB_ECOLI          GLIAVVSVKVPDPKFSSQTKDKLVSSEVKSAVEQQMNELLAEYLLENPTDAKIVVGKIID 377
sp|P9GW45|GYRB_MYCTU          GLAAVISVKVSEPQFEGQTKTKLGNTEVKSFVQKVCNEQLTHWFEANPTDAKVVVNKAVS 412
sp|Q59533|GYRB_MYCLE          GLAAVISVKVSEPQFEGQTKTKLGNTEVKSFVQRVCNEQLIHWFEANPVDAKAVVNKAIS 415
                              **:**:****::*:*::***:**::*****:*:µ::**:*µ::::::**µ***µ**:*:µ:

                                                                                  <--------
sp|P0AES6|GYRB_ECOLI          AARAREAARRAREMTRRKGALDLAGLPGKLADCQERDPALSELYLVEGDSAGGSAKQGRN 437
sp|P9GW45|GYRB_MYCTU          SAQARIAARKARELVRRKSATDIGGLPGKLADCRSTDPRKSELYVVEGDSAGGSAKSGRD 472
sp|Q59533|GYRB_MYCLE          SAQARIAARKARELVRRKSATDLGGLPGKLADCRSTDPRSSELYVVEGDSAGGSAKSGRD 475
                              :*:**:***:***::***:*:*µ:**********::**:µ*****************:**:

                                          QRDR
                              ---------------->
sp|P0AES6|GYRB_ECOLI          RKNQAILPLKGKILNVEKARFDKMLSSQEVATLITALGCGIGRDEYNPDKLRYHSIIIMT 497
sp|P9GW45|GYRB_MYCTU          SMFQAILPLRGKIINVEKARIDRVLKNTEVQAIITALGTGIH-DEFDIGKLRYHKIVLMA 531
sp|Q59533|GYRB_MYCLE          SMFQAILPLRGKIINVEKARIDRVLKNTEVQAIITALGTGIH-DEFDISRLRYHKIVLMA 534
                              :::******:***:******:*::*:::**:::*****:**::**:::µµ****:*::*:
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```
sp|P0AES6|GYRB_ECOLI        DADVDGSHIRTLLLTFFYRQMPEIVERGHVYIAQPPLYKVKKGKQEQYIKDDEAMDQYQI 557
SP|P9GW45|GYRB_MYCTU        DADVDGQHISTLLLTLLFRFMRPLIENGHVFLAQPPLYKLKWQRSDPEFAYSDRERDGLL 591
sp|Q59533|GYRB_MYCLE        DADVDGQHISTLLLTLLFRFMRPLIEHGYVFLAQPPLYKLKWQRMDPEFAYSDSERDGLL 594
                            ******:**:*****:::*:*::::***µ*::********:*:::µ:::::::::::::

sp|P0AES6|GYRB_ECOLI        SIALDGATLHTNASAPALAGEALEKLVSEYNATQKMINRMERRYPKAMLKELIYQPTLTE 617
SP|P9GW45|GYRB_MYCTU        EAGLKAG---------------------------------------------------- 598
sp|Q59533|GYRB_MYCLE        ETGLKLG---------------------------------------------------- 601
                            :µ:*:µ::::::::::::::::::::::::::::::::::::::::::::::::::::::

sp|P0AES6|GYRB_ECOLI        ADLSDEQTVTRWVNALVSELNDKEQHGSQWKFDVHTNAEQNLFEPIVRVRTHGVDTDYPL 677
SP|P9GW45|GYRB_MYCTU        ---------------------------------------------------------- 598
sp|Q59533|GYRB_MYCLE        ---------------------------------------------------------- 601
                            ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::

sp|P0AES6|GYRB_ECOLI        DHEFITGGEYRRICTLGEKLRGLLEEDAFIERGERRQPVASFEQALDWLVKESRRGLSIQ 737
SP|P9GW45|GYRB_MYCTU        ----------------------------------------------KKINKEDGIQ 608
sp|Q59533|GYRB_MYCLE        ----------------------------------------------KKINKEDGIQ 611
                            ::::::::::::::::::::::::::::::::::::::::::::::*:::::::**

sp|P0AES6|GYRB_ECOLI        RYKGLGEMNPEQLWETTMDPESRRMLRVTVKDAIAADQLFTTLMGDAVEPRRAFIEENAL 797
SP|P9GW45|GYRB_MYCTU        RYKGLGEMDAKELWETTMDPSVRVLRQVTLDDAAAADELFSILMGEDVDARRSFITRNAK 668
sp|Q59533|GYRB_MYCLE        RYKGLGEMDAKELWETTMDPSVRVLRQVTLDDAAAADELFSILMGEDVDARRSFITRNAK 671
                            ********:::********::*::::**::**:***:**::***::*::**:**::**:
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sp|P0AES6|GYRB_ECOLI            KAANIDI 804
SP|P9GW45|GYRB_MYCTU            DVRFLDV 675
sp|Q59533|GYRB_MYCLE            DVRFLDV 678
                                :::::*:
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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.

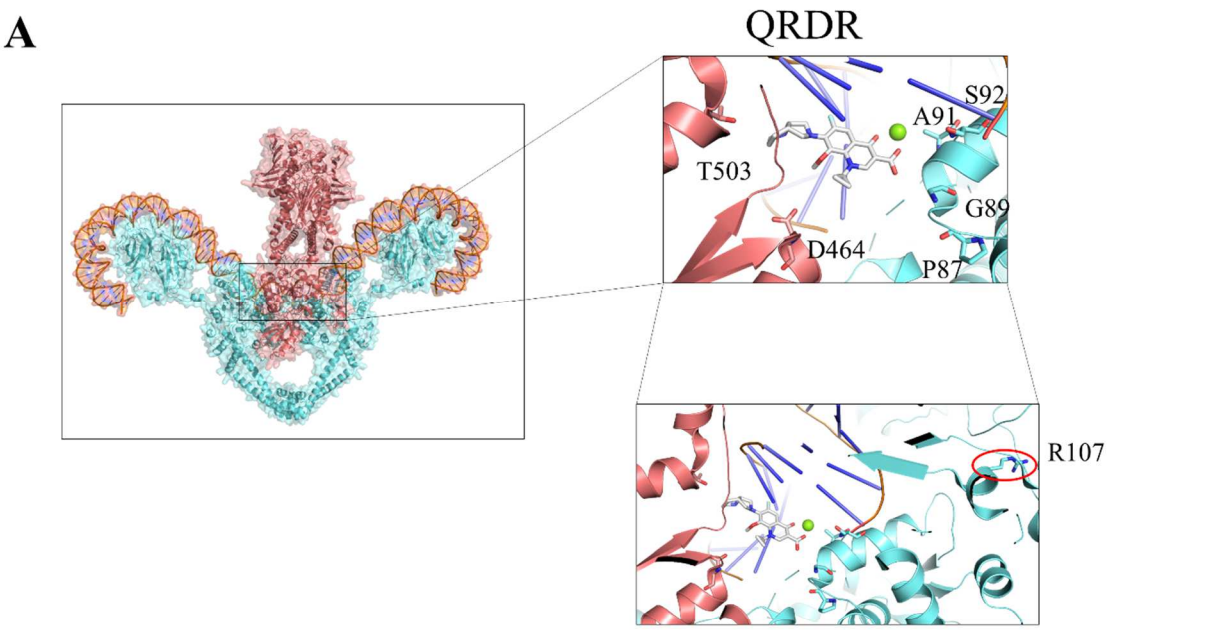
A
QRDR
S92
A91
T503
G89
D464
P87
R107
B
GyrA
V311
GyrA I431
GyrA G695
C
GyrB V214

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

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

[a] P9WG47

[b] P9WG47

[c] Q57532

[d] not applicable because mutations are located outside the intein

[e] not applicable because mutations are located inside the intein

[f] 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.

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

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.

[a] P0AES6

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

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

[d] Zhou et al. [51]

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

[f] 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[a]	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[b]	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]
Total number of isolates	**1985 MB/76PB 2134 MB/ 94PB/ 53 BT/ 8TT/ 8BL/ 3BB/ 4 healed fibrosed and 1 axonopathy**	**2884**			**115**	**1110**	**3/24**	

DST: Drug Susceptibility Testing; NA: not applicable; ND: no data; PCR seq: PCR and DNA sequencing; WGS: whole genome sequencing; DNA strip test: GenoType LepraeDR® (Hain, Lifescience); QRDR_A: QRDR gyrase A; QRDR_AB: QRDR gyrase A and B.

[a] number of strains with their gyrase genes sequenced (*gyrA*, *gyrB* or *gyrA* and *gyrB*).

[b] BT: borderline tuberculoid; TT: tuberculoid; BL: borderline lepromatous; BB: mid borderline.

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

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

GyrA	**A91V**	**A91V**	1	ND	yes	[53]
GyrA	**A91P**	**A91P**	2	no	no	[29]
GyrA	A91T	A91T	3[b]	not for leprosy	yes	[38]
GyrA	**S92A**	**S92A**	6[c]	not for leprosy	yes	[38]
GyrA	**S92A**	**S92A**	1	ND	yes	[52]
GyrA	L97P	L97P	2	ND	yes for 1	[56]
GyrA	R107L[d]	R107L	1	under treatment	no	[39]
GyrA	V731I	V311I	1	ND	ND	[26]
GyrA	G362D	[e]	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]
Multiple substitutions in GyrA						
GyrA	**G89C** + **A91V**	**G89C** + **A91V**	2	ND	yes	[32]
GyrA	A91T + **S92A**	A91T + **S92A**	1	no	yes	[38]
Single substitution in GyrB						
GyrB	V214G[f]	V214G	1	ND	ND	[26]
GyrB	V214G	V214G	1	no	ND	[40]
GyrB	D205N	**D464N**	1	ND	yes	[3]
GyrB	D205N	**D464N**	1	yes	yes	[41]
GyrB	**D464N**[g]	**D464N**	1	ND	ND	[26]
GyrB	T503I[h]	T503I	1	ND	ND	[26]
Multiple substitutions in GyrA and GyrB						
GyrA-B	**A91V** (A) **+ D464N (B)**	**A91V** (A) **+ D464N (B)**	1	ND	ND	[26]
GyrA-B	V731I (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]

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.

[a] associated with GyrA A91V substitution.

[b] 1 associated with GyrA S92A substitution.

[c] 1 associated with GyrA A91T substitution.

[d] 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]

[e] 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.

[f] associated with GyrA I431T substitution.

[g] associated with GyrA A91V substitution.

[h] 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_{50} (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]
G89C	WT	160	30	[16]
G89C	WT	nd	22.6+/-2.9	[58]
A91V[a]	WT	80	25	[16]
A91V[a]	WT	nd	2.1+/-0.1	[58]
D95G[b]	WT	161.2+/-44.2	21.5+/-4.7	[57]
D95G[b]	WT	nd	12.2+/-1.3	[58]
D95N[b]	WT	262.3+/-105.8	34.7+/-3.1	[57]
WT	*D205N*	20	6	[16]
WT	**D464N**	53.9+/-9	4.1+/-0.4	[48]
WT	**N502D**[b]	106.6+/-25.1	17.8+/-2.6	[48]
WT	**E504V**[b]	34.6+/-4.3	13.9+/-0.6	[48]

IC_{50}, 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.

[a] the impact of this substitution was also demonstrated using the mouse footpad technique. [7,13,15,21–23]

[b] substitution never described in a clinical isolate of *M. leprae*.