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A systematic review of gyrase mutations associated with fluoroquinolone-resistant *Mycobacterium tuberculosis* and a proposed gyrase numbering system

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Fluoroquinolone resistance in *Mycobacterium tuberculosis* has become increasingly important. A review of mutations in DNA gyrase, the fluoroquinolone target, is needed to improve the molecular detection of resistance. We performed a systematic review of studies reporting mutations in DNA gyrase genes in clinical *M. tuberculosis* isolates. From 42 studies that met inclusion criteria, 1220 fluoroquinolone-resistant *M. tuberculosis* isolates underwent sequencing of the quinolone resistance-determining region (QRDR) of *gyrA*; 780 (64%) had mutations. The QRDR of *gyrB* was sequenced in 534 resistant isolates; 17 (3%) had mutations. Mutations at *gyrA* codons 90, 91 or 94 were present in 654/1220 (54%) resistant isolates. Four different GyrB numbering systems were reported, resulting in mutation location discrepancies. We propose a consensus numbering system. Most fluoroquinolone-resistant *M. tuberculosis* isolates had mutations in DNA gyrase, but a substantial proportion did not. The proposed consensus numbering system can improve molecular detection of resistance and identification of novel mutations.

Keywords: *gyrA*, *gyrB*, QRDRs, *M. tuberculosis*

Introduction

Fluoroquinolones play an increasingly important role in the treatment of tuberculosis (TB). They are used to treat multidrug-resistant tuberculosis (MDR-TB; defined as resistance to at least isoniazid and rifampicin) and are also recommended to treat drug-susceptible TB in patients with intolerance to first-line antibiotics.[1] Fluoroquinolones kill bacteria by altering DNA gyrase and DNA topoisomerase IV. Since *Mycobacterium tuberculosis* does not have topoisomerase IV, fluoroquinolones target DNA gyrase in *M. tuberculosis*.[2] DNA gyrase consists of two A and two B subunits encoded by the *gyrA* and *gyrB* genes, respectively.[3] The quinolone resistance-determining region (QRDR) is comprised of conserved areas within *gyrA* and *gyrB* in which mutations conferring fluoroquinolone resistance have been reported in most bacterial species, including *M. tuberculosis*.[3–5]

There are several fluoroquinolone resistance mechanisms in bacteria. Although the primary fluoroquinolone resistance mechanism in *M. tuberculosis* is related to mutations in the QRDR of DNA gyrase genes,[6–12] fluoroquinolone resistance can also be conferred by increased fluoroquinolone efflux or DNA mimicry.[13–15] However, the latter two mechanisms have been described only in laboratory strains, not clinical *M. tuberculosis* isolates. In many studies, more than 90% of fluoroquinolone-resistant *M. tuberculosis* strains have mutations in the QRDR of *gyrA* or *gyrB*,[16–27] so we focused our review on mutations in DNA gyrase.

Conventional phenotypic *M. tuberculosis* drug susceptibility testing (DST) methods for fluoroquinolones require several weeks to complete and are not standardized. Therefore, molecular diagnostic tests for rapid detection of fluoroquinolone resistance at the point-of-care are urgently needed. In addition to fluoroquinolone mono-resistant *M. tuberculosis* isolates, such tests could be used to rapidly identify extensively

drug-resistant tuberculosis (XDR-TB; defined as resistance to at least isoniazid and rifampicin plus a fluoroquinolone and a second-line injectable agent such as capreomycin, amikacin and kanamycin).[28–30] A line probe assay has been developed to detect fluoroquinolone-resistance in *M. tuberculosis*, but its sensitivity ranges from 75.6% to 89.5% when compared with conventional phenotypic susceptibility methods,[19,31,32] and 87% to 100% when compared with DNA sequencing.[31,33]

The purpose of this review was to characterize all DNA gyrase gene mutations described in *M. tuberculosis* clinical strains and to distinguish those associated with fluoroquinolone resistance and those reported in fluoroquinolone-susceptible strains. During the review process we found four different numbering systems used for the GyrB subunit, resulting in discrepancies regarding the location of resistance mutations. Some authors used the *Escherichia coli* numbering system; others used one of three *M. tuberculosis* numbering systems, which differ according to the position of the start codon.[2,3,34] Most authors did not specify the numbering system used. Even the comprehensive compilation of mutations in the online database http://www.tbdreamdb.com,[35] which focuses on the QRDR and uses one consistent numbering system, classifies some mutations as inside the QRDR of GyrB when they are indeed outside the QRDR. The different numbering systems make it difficult to confirm previously described mutations and clearly identify novel mutations. We therefore sought to clarify the location of these mutations.

To our knowledge this is the most extensive review to date of mutations described in the *gyrA* and *gyrB* genes of fluoroquinolone-resistant and -susceptible strains of *M. tuberculosis*. It is also the first work to propose a standard numbering system of *M. tuberculosis* GyrA and GyrB, which will allow for accurate comparison of resistance mutations by laboratories around the world.

Methods

Definitions

Mutations were defined as nucleotide base-pair changes that resulted in substitutions in amino acids (i.e. non-synonymous), regardless of whether the mutation was reported in a fluoroquinolone-resistant or -susceptible *M. tuberculosis* isolate. Of those mutations reported in fluoroquinolone-resistant isolates, we distinguished between mutations documented to confer fluoroquinolone resistance by biochemical or genetic experiments and those without such evidence.[18,36] Polymorphisms were defined as non-synonymous nucleotide base-pair changes known to not be associated with or confer fluoroquinolone resistance. Base-pair changes that did not result in a change in amino acid (i.e. synonymous) were not included in this review. The three-letter abbreviation nomenclature was used to represent amino acids. Substitutions were noted as follows: Xxx##Yyy, where Xxx represents the wild-type amino acid, ## the codon number and Yyy the substituted amino acid.

Search strategy

A computerized search identified peer-reviewed primary research studies reporting fluoroquinolone-resistant or fluoroquinolone-susceptible isolates of *M. tuberculosis* in which mutations in DNA gyrase genes were identified. The search was limited to studies in English published between 1 January 1990 and 30 June 2010. Figure S1 (available as Supplementary data at *JAC* Online) illustrates the study selection methodology. Full text articles were screened using the Medical Literature Analysis and Retrieval System Online (MEDLINE) using the keywords 'fluoroquinolone resistance', '*M. tuberculosis*', '*gyrA*', '*gyrB*', 'DNA gyrase', 'mutations', 'drug resistance', 'fluoroquinolone susceptibility', 'second-line drug resistance', 'quinolone resistant' and 'ofloxacin resistance' in different combinations.

The inclusion criteria consisted of (i) publications in which genotypic susceptibility methods were compared with a solid or liquid-based phenotypic resistance reference standard and (ii) DNA gyrase gene mutations were identified in *M. tuberculosis* isolates obtained from human clinical specimens.

Papers were excluded if they were reviews, letters, duplicates or if the title indicated that the study was not relevant to our study. The online database http://www.tbdreamdb.com[35] was excluded because it is a compilation of mutations previously reported in the literature rather than a primary source document. Abstracts of the remaining papers were reviewed and studies with irrelevant content were excluded. If the abstract did not provide enough information to include or exclude the article, the entire article was reviewed. Articles were also excluded if they lacked data on amino acid changes or phenotypic susceptibility testing. The bibliographies of the included publications were reviewed, and additional articles not previously identified were added as appropriate.

Data acquisition

Data abstracted from journal articles that met the inclusion criteria were organized in three ways: (i) all mutations reported in *gyrA*, (ii) all mutations reported in *gyrB* and (iii) all combinations of mutations (in *gyrA* and/or *gyrB*) reported in a single *M. tuberculosis* isolate. Although substitutions Glu21Gln, Ser95Thr and Gly668Asp in GyrA result in amino acid changes, they were omitted from the summary tables because they are common polymorphisms that do not correlate with drug resistance.[3,37]

When more than one mutation was observed in one strain (double or triple mutation), we noted two scenarios: (i) either 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. Mutations that were never observed independently of one another are noted in the tables. This process was designed to capture every mutation without undermining the potential effect that combinations of mutations may have on fluoroquinolone resistance.

For this review, all of the substitutions in GyrB were standardized to the re-annotated genome numbering system of *M. tuberculosis* GyrB, where the QRDR of *M. tuberculosis* GyrB ranges from codon 461 to 499.[34] Regarding the QRDR of GyrA, some publications used the *E. coli* numbering system to describe substitution location.[38–41] In this systematic review, all substitution locations in GyrA were standardized to the genome *M. tuberculosis* numbering system, in which the QRDR of GyrA ranges from codon 74 to 113.[3]

We reported the number of clinical isolates tested, the region sequenced (entire *gyrA* or *gyrB* genes or only the QRDRs of *gyrA* or *gyrB*), along with the genotypic and phenotypic susceptibility methods used to determine fluoroquinolone resistance for each study. The number of isolates containing a specific mutation was determined and the fluoroquinolone MIC associated with this mutation was included if available. Fluoroquinolone activity (measured as 50% inhibitory concentration) against *M. tuberculosis* with specific DNA gyrase mutants was also reviewed.

Quality control

Two authors (F. M. and A. W. K.) independently reviewed and abstracted the data. The data were reviewed for accuracy and compared with particular attention to the numbering systems used. Two additional authors (Y. F. van der H. and A. A.) adjudicated differences between the authors and reviewed the data for accuracy.

Results

Numbering systems for M. tuberculosis GyrA and GyrB subunits

GyrA

The first studies investigating the molecular basis of fluoroquinolone resistance in *M. tuberculosis* were based on *E. coli*[39] or *M. tuberculosis gyrA*[3] gene sequences. Since the GyrA QRDR is located at the N-terminal part of the GyrA subunit and the *M. tuberculosis* GyrA start codon is seven amino acids before the *E. coli* GyrA (Figure S2, available as Supplementary data at *JAC* Online), the amino acids at positions 88, 90, 91 and 94 in the *M. tuberculosis* numbering system correspond to the amino acids at positions 81, 83, 84 and 87 in the *E. coli* numbering system, respectively. When these differences were accounted for, there were no discrepancies in the location of substitutions reported in the GyrA QRDR in any of the studies reviewed.

GyrB

In contrast, four distinct numbering systems have been used to report substitutions in the GyrB QRDR. As a result, in several instances amino acid changes occurring at what was actually the same position were reported as occurring at different positions. For example, substitution Asp472Ala[33] (2000; Table 1) is the same as substitution Asp500Ala[42] (1998; Table 1), but the authors used different numbering systems. Codons 472 and 500 both correspond to position 426 in the *E. coli* numbering system (Table 1 and Figure S3, available as Supplementary data at *JAC* Online). Similarly, substitution Asn533Thr[26] (1994; Table 1) is the same as Asn538Thr[42] (1998; Table 1); both correspond to codon 464 in the *E. coli* numbering system. Additionally, discrepancies in the numbering system resulted in differences in classifying mutations as being inside or outside of the QRDR of GyrB. For example, mutations at codons 500 and 501 in the proposed numbering system (2002; Table 1) were designated as inside the QRDR in three studies, but are definitely located outside the QRDR of GyrB.[33,42,43]

Most discrepancies arose from a lack of consensus regarding the *gyrB* start codon. The first sequence of *M. tuberculosis gyrB* (published in 1994) described the GyrB QRDR extending from codon 495 to 533[3] (see gi|1107468|gb|AAA83016.1| in Figure S3). This numbering system was based on the GyrB *E. coli* sequence. This numbering system was used for the first studies reporting fluoroquinolone resistance in *M. tuberculosis*.[36] When the entire genome of *M. tuberculosis* was published in 1998, the start codon of *gyrB* was 28 codons upstream of the codon that had been used earlier. Due to the absence of some amino acids in *E. coli* that are present in *M. tuberculosis*, the

Table 1. Comparison of the three GyrB numbering systems in *M. tuberculosis* described in the literature, the corresponding region in *E. coli* and the proposed consensus numbering system

		M. tuberculosis GyrB sequence (year of publication)			
Substitution observed	*E. coli*[a]	1994[b]	1998[c]	2000[d]	2002[e] proposed numbering system
Arg→Cys	411	480	485	457	446
Ser→Phe	412	481	486	458	447
Asp→Ala	426	495	500	472	461
Asp→Asn	426	495	500	472	461
Asp→His	426	495	500	472	461
Gly→Ala	435	504	509	481	470
Asp→Ala	459	528	533	505	494
Asn→Asp	464	533	538	510	499
Asn→Lys	464	533	538	510	499
Asn→Thr	464	533	538	510	499
Thr→Asn	465	534	539	511	500
Thr→Pro	465	534	539	511	500
Glu→Asp	466	535	540	512	501
Glu→Val	466	535	540	512	501
Ala→Thr	469	538	543	515	504
Ala→Val	469	538	543	515	504
Gln→His	503	572	577	549	538
Deletion	Δ609+610	Δ678+679	Δ683+684	ND	Δ644+645

ND, not determined.
The QRDR of GyrB is enclosed in the bold box and ranges from codon 461 to codon 499 in the proposed *M. tuberculosis* GyrB sequence.[34]
[a]P0AES6.
[b]AAA83016.1.[3]
[c]CAB02426.1.[2]
[d]Zhou *et al.*[44]
[e]P0C5C5|1-675.[34]

first codon of the GyrB QRDR was five codons upstream of the codon that had been used earlier; the QRDR therefore ranged from amino acid 500 to 538[2] (see gi|1552558|emb|CAB02426.1 in Figure S3). Of the studies reviewed, most authors used the 500–538 numbering system to identify mutation location. However, the first *gyrB* mutations described in clinical *M. tuberculosis* strains were identified using yet another numbering system, which did not correspond to any of the previously published sequences. The publications using this numbering system did not provide references to justify this numbering sytem.[7,18,21,33,43–45] In 2002, a re-annotation of the *M. tuberculosis* genome proposed a new start codon for *gyrB*.[34] This numbering system is closer to the *E. coli* numbering system, thus shifting the QRDR to residues 461–499[34] (see P0C5C5|1-675 in Figure S3). This latter proposed numbering system appears to be the most accurate. Despite the absence of experiments defining the *gyrB* start codon, the alignment of *M. tuberculosis* GyrB sequence and 50 bacterial species (other than *M. tuberculosis*) from five phyla whose sequences were obtained from the SWISS-PROT/TrEMBL (http://expasy.org/sprot) and NCBI (http://www.ncbi.nlm.nih.gov) databases demonstrated that the start codon chosen by Camus *et al.*[34] was the most accurate (C. Mayer, personal data). This is the numbering system that we propose future studies use as the reference regarding fluoroquinolone resistance in *M. tuberculosis*.

Table 1 compares the three *M. tuberculosis* GyrB numbering systems used in the literature as well as the *E. coli* GyrB numbering system. The consensus numbering system that we propose is also included.

Findings

Forty-two publications met the inclusion criteria. From these studies, a total of 2482 *M. tuberculosis* isolates were assessed for genotypic mutations: 1220 (49%) were phenotypically fluoroquinolone resistant and 1262 (51%) were fluoroquinolone susceptible. The phenotypic and genotypic resistance methods used and the regions of *M. tuberculosis* DNA sequenced are provided in Table 2. Twenty-seven studies sequenced the QRDR of *gyrA*, 13 sequenced the QRDR of both *gyrA* and *gyrB*, 1 sequenced the entire *gyrA* and 1 sequenced the entire *gyrA* and *gyrB* genes (Table 2).

There were *gyrA* or *gyrB* mutations identified in 806/1220 (66%) phenotypically resistant isolates and 19/1262 (2%) phenotypically susceptible isolates. Specific mutations identified in each *gyrA* and *gyrB* gene are described in the following sections.

Among the 2482 isolates tested, 44 distinct mutations were identified. Of the 44 mutations, 26 (59%) were in GyrA and 18 (41%) in GyrB; they occurred at 24 different codons. Of the 44 mutations, 34 (77%) occurred in phenotypically resistant *M. tuberculosis* isolates, 5 (11%) in phenotypically susceptible isolates and 5 (11%) in both resistant and susceptible isolates. These findings are discussed in greater detail below and in Tables 3–5.

Mutations in gyrA

Of the 26 GyrA mutations, 21 (81%) were inside the QRDR and 5 (19%) were outside the QRDR, including 1 (4%) that was in the putative promoter (Table 3). Of the 1220 fluoroquinolone-resistant isolates sequenced, 780 (64%) had mutations inside the QRDR of GyrA and 6 (0.5%) had mutations outside of the QRDR of GyrA (Table 3). Substitutions at codon 94 were the most prevalent; they were reported in 37% of the fluoroquinolone-resistant strains. Various substitutions were reported at codon 94 in GyrA: aspartic acid (Asp) was replaced by alanine (Ala), asparagine (Asn), glycine (Gly), histidine (His), phenylalanine (Phe), tyrosine (Tyr) or valine (Val). The Asp94Gly substitution in GyrA occurred most frequently; it was reported in 234 (19%) resistant isolates. Overall, mutations at codons 90, 91 or 94 were reported in 654 (54%) of the 1220 fluoroquinolone-resistant isolates, with substitutions at codons 90 and 91 occurring in 13% and 4% of the isolates, respectively.

Of the 1262 fluoroquinolone-susceptible isolates studied for GyrA, 14 (1%) had mutations inside the QRDR of GyrA and 2 had a substitution in the putative promoter of GyrA[26] (Table 3).

Mutations in gyrB

Of the 18 GyrB mutations, 8 (44%) were inside the QRDR, 9 (50%) were outside the QRDR and 1 (6%) was a deletion also outside the QRDR (Table 4). Although all 42 studies sequenced the GyrA QRDR, only 14 studies sequenced GyrB (mostly the QRDR) (Table 2). Of the 534 resistant isolates studied for *gyrB*, 17 (3%) harboured mutations in the QRDR and 13 (2%) harboured mutations outside of the QRDR. Ten (2%) isolates had a substitution at codon 499; Asn499Asp (in the proposed numbering system, 2002; Table 1) was reported in 4 (0.7%) of the 10 resistant isolates. In one case, deletion of codons 644 and 645 in GyrB was reported in a fluoroquinolone-resistant strain.[26] Among the 377 susceptible strains, there was one substitution reported in GyrB (Ser447Phe in the proposed numbering system; Table 1) outside the QRDR. Codon mutations and associated MICs are presented in Table 6.

Multiple mutations and heteroresistance

Several studies reported multiple mutations in *gyrA*, *gyrB* or in both *gyrA* and *gyrB* (Table 5), with up to five mutations in the same strain.[42] Double mutations in *gyrA* have often been described as two mutations in the same *gyrA* allele and have been associated with high-level resistance.[36] They may result from a two-step selection of fluoroquinolone-resistant mutants.[18] The combination of Ala90Val with Asp94Gly is frequently associated with high-level resistance and was reported in 34 (3%) of the fluoroquinolone-resistant isolates.[19,21,33,42,46,47] The combination of Thr80Ala with Ala90Gly, which was demonstrated to confer fluoroquinolone susceptibility[18] was the most frequent double mutation reported among fluoroquinolone-susceptible isolates [6 (0.5%) isolates] (Tables 5–7).

In several studies, sequencing showed heterogeneous peaks in up to 31.2% of the isolates.[19,42,48] Such heteroresistance refers to the concomitant presence of susceptible and resistant bacilli in the same clinical specimen or the presence of several clones, each harbouring a distinct mutation.[49] It probably results from a strain splitting into two or more bacterial clones or less likely from infection by two different strains.[50]

Mutations that conferred fluoroquinolone resistance (rather than simply being present in fluoroquinolone-resistant

Table 2. Clinical *M. tuberculosis* isolates studied: fluoroquinolone susceptibility and molecular detection methods used in each primary study included in the review

Type of collection	No. of FQR	No. of FQS	Susceptibility method (OFX breakpoints)	Molecular detection method	DNA region studied[a]	Percentage FQR with mutations	Study reference
ND	4	0	PMS on 7H11 (2 mg/L)	PCR seq	QRDR_A	100	25
ND	8	1	ACM on LJ (2 mg/L)	PCR seq	QRDR_A	100	27
ND	87	0	ACM on LJ	PCR seq	QRDR_A	100	46
HIV-infected TB cases	1	0	MIC in liquid media (7H12+Bactec)	PCR seq	QRDR_A	100	60
MDR	52	55	ACM on LJ (2 mg/L)	microchips	QRDR_A	98	17
ND	71	47	ACM on LJ (8 mg/L)	PCR seq	QRDR_A	97.2	24
XDR	26	0	PML (MGIT 960+Bactec 460)	PCR seq	QRDR_A	96.2	22
MDR	35	108	ACM on LJ (2.4 mg/L)+PML (MGIT960)	PCR seq	QRDR_A	94.3	20
MDR	11	43	PMS on 7H11 (2 mg/L)	MAS-PCR	QRDR_A	91	53
Fluoroquinolone, AMK, CAP and/or EMB resistance	32	74	PML (MGIT 960)+PMS on LJ (2 mg/L)	PCR seq+MTBDRsl	QRDR_A	90.6	19
ND	19	9	MIC on 7H11 (2 mg/L)	PCR seq+line probe assay	QRDR_A	89.5	31
ND	30	0	PMS on LJ (2 mg/L)	PCR seq	QRDR_A	86.7	61
XDR	13	0	MIC in solid medium (5 mg/L)	PCR seq	QRDR_A	85	62
ND	13	6	growth inhibition in liquid culture (ID50)	PCR seq	QRDR_A	84.6	63
Drug resistant	71	179	ACM (4.8 mg/L)	PCR-SSCP/MPAC+PCR seq	QRDR_A	78	54
ND	42	40	PMS on LJ (2 mg/L)	LNA-PCR	QRDR_A	76.2	48
ND	10	92	disc proportion method	pyrosequencing	QRDR_A	70	55
ND	110	0	MIC (5 mg/L)	PCR seq, DHPLC	QRDR_A	67.5	47
ND	12	0	PMS (2 mg/L)	PCR seq	QRDR_A	66.7	12
ND	55	83	ACM on LJ (2 mg/L)	PCR seq	QRDR_A	58	6
ND	4	0	PMS on LJ+MIC in solid medium (2 mg/L)	PCR seq	QRDR_A	50	11
ND	1	0	PMS on LJ (2 mg/L)	PCR seq	QRDR_A	ND	39
ND	0	16	PML in 7H9	PCR seq	QRDR_A	ND	40
ND	2	0	MIC on LJ	PCR seq	QRDR_A	ND	64
ND	2	0	MIC on 7H10 (2 mg/L)	PCR seq	QRDR_A	ND	38
ND	3	3	MIC (2 mg/L)	non-radioactive PCR-SSCP, PCR seq	QRDR_A	ND	41
ND	16	0	PML, Bactec 460	PCR-SSCP, PCR seq	QRDR_A	ND	65
ND	15	39	PML (Bactec) (2 mg/L)	non-radioactive PCR-SSCP, PCR seq	QRDR_AB	100	3
ND	4	4	MIC in 7H11 (2 mg/L)	PCR seq	QRDR_AB	100	18
ND	24	28	PMS on LJ (2 mg/L)	PCR seq+MTBDRsl	QRDR_AB	100	33
MDR	26	49	PMS (2 mg/L)	PCR seq	QRDR_AB	96	16
FQR and MDR	48	23	ACM (2 mg/L)	PCR seq	QRDR_AB	91.7	21
Consecutive FQR	109	0	PMS on LJ (2 mg/L)	PCR seq	QRDR_AB	82.6	42
ND	60	2	ACM on 7H11	PCR seq	QRDR_AB	73.3	43

Continued

Table 2. *Continued*

Type of collection	No. of FQR	No. of FQS	Susceptibility method (OFX breakpoints)	Molecular detection method	DNA region studied[a]	Percentage FQR with mutations	Study reference
FQR	35	0	PMS and ACM	PCR seq+SSCP	QRDR_AB	60	8
ND	10	131	MIC on 7H11 (2 mg/L)	PCR seq	QRDR_AB	50	9
ND	14	28	MIC in solid medium (2 mg/L)	PCR seq	QRDR_AB	42.8	66
ND	68	0	PMS (2 mg/L)	PCR seq	QRDR_AB	10.3	10
ND	48	52	Etest (4 mg/L)	PCR seq	QRDR_AB	2	7
ND	1	0	MIC in 7H9 (2 mg/L)	PCR seq	QRDR_AB	ND	45
ND	3	135	PMS on 7H10	PCR seq	entire *gyrA*	100	23
ND	25	15	PMS on 7H11 (2 mg/L)+REMA	PCR seq	entire *gyrA* and *gyrB*	100	26
Total number of isolates	1220	1262					

ACM, absolute concentration method on Lowenstein–Jensen; AMK, amikacin; CAP, capreomycin; EMB, ethambutol; MIC, MIC by dilution method in solid or liquid medium; ND, no data; No. of FQR, number of fluoroquinolone-resistant strains as defined in the study; No. of FQS, number of fluoroquinolone-susceptible strains as defined in the study; OFX, ofloxacin; PCR seq, PCR and DNA sequencing; PML, proportion method in liquid medium (Bactec 460 or MGIT 960); PMS, proportion method on solid medium (Lowenstein–Jensen, 7H10 or 7H11); QRDR_A, QRDR gyrase A; QRDR_AB, QRDR gyrase A and B; REMA, resazurin microtitre assay.
[a]Some studies that sequenced the QRDR of *gyrA* also included some codons near the QRDR, but not the entire *gyrA*.

M. tuberculosis isolates) were identified based on biochemical studies demonstrating that the altered DNA gyrase subunit was resistant to fluoroquinolone inhibition. Table 7 lists the mutations that have been demonstrated to confer fluoroquinolone resistance.

Discussion

Because *M. tuberculosis* is a slow-growing bacterium, the detection of drug resistance by traditional methods requires 5–12 weeks. Several studies have demonstrated that molecular tests facilitate rapid diagnosis of resistance in *M. tuberculosis*, including MDR-TB and XDR-TB, which can occur 2–3 months faster than with conventional susceptibility testing.[51] To maximize the sensitivity and specificity of molecular testing for fluoroquinolone resistance in *M. tuberculosis*, it is critical to have comprehensive knowledge of the fluoroquinolone resistance mutations.

Because the primary mechanism of fluoroquinolone resistance in *M. tuberculosis* occurs via modification of DNA gyrase, it is important to (i) summarize all mutations described in DNA gyrase genes of *M. tuberculosis* and (ii) clarify the role of these mutations in fluoroquinolone resistance.

Our review differs from the compilation of mutations in tbdream,[35] in that our review includes all mutations reported outside the QRDR of *gyrA* and *gyrB* (including heterogeneity at codons), addresses the four different *gyrB* numbering systems, provides the prevalence of common resistance mutations, notes which mutations have been demonstrated to confer resistance and provides the range of MICs reported for mutations at each codon.

We needed first to clarify the discrepancies of the GyrB numbering system to be able to differentiate novel from previously described mutations. We propose that the GyrB sequence that was re-annotated in 2002[34] should be used as the reference sequence (Table 1 and Figure S3).

Having clarified the numbering system of GyrB, we reviewed all the mutations described in the DNA gyrase genes of clinical *M. tuberculosis* isolates.

Mutations at GyrA codons 90, 91 or 94 were most common,[3,6–8,38,52] and seen in 654 (54%) resistant isolates. Although a majority of fluoroquinolone-resistant *M. tuberculosis* isolates had mutations in these codons, a substantial proportion did not. This suggests that the sensitivity of line-probe assays or other molecular diagnostic tests that focus on these codons could have low sensitivity. However, the isolates in this systematic review, although representing all fluoroquinolone-resistant *M. tuberculosis* isolates reported to date, and encompassing multiple countries of the world (e.g. China, France, India, Russia, the USA, Uzbekistan and Vietnam), may differ from population-based studies.

Based on our review, the most widely used molecular method to detect fluoroquinolone resistance in *M. tuberculosis* was PCR and DNA sequencing (Table 2). Other, more rapid testing methods included multiplex allele-specific PCR (MAS-PCR),[53] PCR single-stranded conformation polymorphism/multiplex PCR amplimer conformation (PCR-SSCP/MPAC),[54] pyrosequencing,[55] denaturing HPLC (DHPLC),[47] non-radioactive PCR-SSCP,[3,8,41] locked nucleic acid probe real-time PCR (LNA-PCR)[48] and

Table 3. GyrA substitutions reported in fluoroquinolone-resistant and -susceptible clinical *M. tuberculosis* isolates

	Study reference	
Substitution[a]	fluoroquinolone-resistant isolates	fluoroquinolone-susceptible isolates
Putative promoter	none	26
Pro8Ala	none	26
Arg68Gly	none	24
His70Arg	43	none
Ala74Ser[b]	46, 47	none
Thr80Ala	18, 25, 33	26, 33, 40
Gly88Ala	9, 33, 42, 48, 64	none
Gly88Cys	8, 17, 21, 33, 54, 60	none
Asp89Asn	26, 48	none
Ala90Glu	33	none
Ala90Gly[b]	33	18, 26, 33
Ala90Leu	11	none
Ala90Val	3, 6, 8, 9, 10, 12, 16, 17, 18, 19, 20, 21, 23, 24, 26, 27, 31, 33, 42, 43, 46, 47, 48, 53, 54, 55, 61, 62, 63, 65, 66	17
Ser91Ala	43	none
Ser91Pro	3, 6, 8, 10, 16, 17, 19, 20, 22, 24, 27, 31, 42, 46, 47, 48, 54, 65	none
Ile92Met	11	none
Asp94Ala	3, 6, 8, 10, 12, 16, 17, 19, 20, 21, 22, 23, 24, 25, 26, 31, 33, 38, 42, 43, 46, 47, 48, 54, 61, 62	54
Asp94Asn	3, 6, 8, 12, 16, 17, 19, 21, 24, 26, 27, 31, 33, 38, 42, 43, 46, 47, 54, 62, 63	none
Asp94Gly	3, 6, 8, 9, 10, 12, 16, 17, 19, 20, 21, 22, 23, 24, 26, 27, 31, 33, 41, 42, 43, 46, 47, 48, 53, 54, 55, 61, 62, 63, 65, 66	none
Asp94His	3, 6, 8, 12, 16, 17, 20, 26, 33, 39, 41, 42, 47, 54, 62, 63	none
Asp94Phe	55	none
Asp94Tyr	3, 6, 9, 12, 16, 17, 19, 20, 21, 26, 41, 42, 46, 47, 48, 54, 61, 62, 63	none
Asp94Val	24	none
Pro102His	none	20
Leu109Val	none	24
Ala126Arg	20, 54	none

The QRDR is enclosed in the bold box and ranges from codon 74 to codon 113.
Substitutions not demonstrated to confer fluoroquinolone resistance in *M. tuberculosis* are in italics.[18]
Substitutions demonstrated to confer fluoroquinolone resistance in *M. tuberculosis* are in bold.[18,64]
Substitutions not italicized or in bold have not been assessed for conferring resistance.
[a]Polymorphisms Glu21Gln, Ser95Thr and Gly668Asp were not included.[37]
[b]Substitutions Ala74Ser and Ala90Gly were never observed independently.[18,26,33,46,47]

GenoType® MTBDRsl (Hain Lifescience) line probe.[19,31,33] These rapid genotypic assays detect fluoroquinolone resistance in isolated cultures of *M. tuberculosis*,[28] and less frequently, directly in respiratory specimens.[51]

Knowledge of all mutations that are involved in fluoroquinolone resistance will help in the design of more sensitive and specific rapid molecular tests. We based our assessment of specific mutations that confer fluoroquinolone resistance on biochemical studies demonstrating that the altered DNA gyrase subunit was resistant to fluoroquinolone inhibition (Table 7). Discrepancies between the results of drug susceptibility testing and biochemical studies can exist since clinical isolates may harbour additional mutations or resistance mechanisms. There are several fluoroquinolone resistance mechanisms in bacteria besides mutations in the *gyrA* and *gyrB* genes. These include mutations in the *parC* and *parE* genes, enhancement of efflux pumps and plasmid-mediated mechanisms such as *qnr* genes encoding pentapeptide repeat proteins, *aac(6′)-Ib-cr* encoding a variant aminoglycoside acetyltransferase and *oqxAB* and *qepA* encoding efflux pumps.[5] For example, biochemical studies on mutant DNA gyrases have demonstrated that the GyrA mutations Thr80Ala and Ala90Gly, and GyrB mutation Ser447Phe do not confer resistance, but these mutations were found in resistant isolates. This may have been because the isolates were misclassified or resistance was conferred by another mechanism (Tables 3, 4 and 7).[18,25,33,42] On the other hand, it is highly surprising to observe that some strains carrying mutations that have previously been demonstrated to confer fluoroquinolone resistance are susceptible to fluoroquinolones (Tables 3 and 7).[17,54] Such discrepancies may be due to misclassification of the strain as susceptible,

Table 4. GyrB substitutions reported in fluoroquinolone-resistant and -susceptible clinical *M. tuberculosis* isolates

Substitution named by author[a]	Substitution in proposed numbering system[b]	Study reference	
		fluoroquinolone-resistant isolates	fluoroquinolone-susceptible isolates
Arg485Cys[c]	Arg446Cys	16	none
Ser486Phe[c]	*Ser447Phe*	42	33
Asp472Aa[d]	Asp461Ala	33, 42	none
Asp500Ala[c]			
Asp495Asn[e]	Asp461Asn	8, 42	none
Asp500Asn[c]			
Asp500His[c]	Asp461His	42	none
Gly509Ala[c]	Gly470Ala[f]	42	none
Asp505Ala[d]	Asp494Ala	7	none
Asn510Asp[d]	**Asn499Asp**	18, 33, 45, 66	none
Asn538Asp[c]			
Asn510Lys[d]	Asn499Lys[f]	21	none
Asn538Thr[c]	Asn499Thr	26, 42	none
Asn533Thr[e]			
Thr511Asn[d]	Thr500Asn	43	none
Thr511Pro[d]	Thr500Pro	33, 42	none
Thr539Pro[c]			
Glu540Asp[c]	Glu501Asp	42	none
Glu540Val[c]	Glu501Val	42	none
Ala543Thr[c]	Ala504Thr	16, 21	none
Ala515Thr[d]			
Ala515Val[d]	Ala504Val	21	none
Gln549His[d]	Gln538His	21	none
Δ678+679[e]	Δ644+645[f]	26	none

The QRDR is enclosed in the bold box.
Substitutions not demonstrated to confer fluoroquinolone resistance in *M. tuberculosis* are in italics.[67]
Substitutions demonstrated to confer fluoroquinolone resistance in *M. tuberculosis* are in bold.[18,64]
[a]See Table 1 for corresponding *M. tuberculosis* numbering systems.
[b]Substitutions are standardized to P0C5C5|1-675,[34] where the QRDR ranges from codon 461 to codon 499.
[c]CAB02426.1.[2]
[d]Zhou *et al*.[44]
[e]AAA83016.1.[3]
[f]Substitutions Gly470Ala, Asn499Lys and Δ644+645 were never observed independently.[21,26,42]

an error in the molecular detection of resistance or the presence of a mixture of strains. For example, in a specimen that harbours mutations leading to GyrA Ala90Val, Asp94Ala and wild-type alleles, results of phenotypic susceptibility testing may have reflected only the wild-type strain.

It is likely that all substitutions observed at codon 94 in GyrA confer fluoroquinolone resistance, even if biochemical tests have not been performed for all substitutions described at this codon. Similarly, it is likely that substitutions at codon 91, reported in fluoroquinolone-resistant strains in 19 studies, are responsible for fluoroquinolone resistance in *M. tuberculosis* (Tables 3 and 6). However, less frequently described mutations such as His70Arg, Asp89Asn and Ile92Met in GyrA and most GyrB mutations (except Ser447Phe and Asn499Asp previously studied) deserve biochemical studies to determine whether or not they confer fluoroquinolone resistance. It is especially important since it has been shown that some mutations may confer high levels of resistance or hypersusceptibility depending on the amino acid substitution.[18] Indeed, it has been previously demonstrated that when alanine at codon 90 of GyrA is replaced by valine, the fluoroquinolone affinity of the resulting enzyme decreases compared with the wild-type, resulting in resistance. However, when it is replaced by glycine the fluoroquinolone affinity of the resulting enzyme increases, resulting in susceptibility (Table 7).[18]

Although the majority of the described mutations occur in the QRDR of GyrA and GyrB (Tables 3–5), some substitutions were described outside of the QRDR. Further studies are necessary to characterize the significance and relevance of these mutations, especially in GyrB, where they are seen more frequently. The GyrB QRDR may need to be redefined if it is determined that substitutions at codons 500–504 (in the proposed numbering system, 2002; Table 1) are implicated in fluoroquinolone resistance.

Table 5. Multiple substitutions in GyrA, GyrB or both GyrA and GyrB reported in fluoroquinolone-resistant and -susceptible clinical *M. tuberculosis* isolates

Substitution[a]	Study reference	
	fluoroquinolone-resistant isolates[b]	fluoroquinolone-susceptible isolates
Multiple substitutions in GyrA		
Ala74Ser[c] + Asp94Gly	**46**, **47**	none
Thr80Ala + Ala90Glu	33	none
Thr80Ala + Ala90Gly[c]	none	18, 26, 33
Thr80Ala + Ala90Gly[c] + Asp94Gly	33	none
Gly88Ala + Ala90Val	**42**	none
Gly88Ala + Asp94Tyr	9	none
Ala90Val + Asp94Ala	23, 42, 43	none
Ala90Val + Pro102His	20, 54	none
Ala90Val + Ser91Pro	17, 46, 47	none
Ala90Val + Asp94Asn	26, 46, 47	none
Ala90Val + Asp94Gly	19, 21, 33, 42, 46, 47	none
Ser91Pro + Asp94Gly	17, 19	none
Ser91Pro + Asp94Gly + Asp94Ala	**42**	none
Asp94Ala + Asp94Tyr	19	none
Asp94Asn + Asp94Gly	19, 21	none
Asp94Asn + Asp94Gly + Asp94Tyr	**21**	none
Asp94Gly + Asp94Ala	**42**	none
Multiple substitutions in GyrB		
Asp461His + Gly470Ala[c]	**42**	
Multiple substitutions in GyrA and GyrB		
Ala90Val + Asp461Ala	42	none
Ala90Val + Asn499Thr	**42**	none
Ala90Val + Asp94Ala + Asn499Thr	**42**	none
Ala90Val + Ser91Pro + Asp94Gly + Asp94Ala + Asn499Thr	**42**	none
Ala90Val + Thr500Pro	**42**	none
Asp94Ala + Asp461Asn	**42**	none
Asp94Gly + Asn499Lys[c]	**21**	none
Asp94Gly + Asn499Thr	**42**	none
Asp94Asn + Ala504Val	21	none
Asp94His + Δ644 + 645[c]	26	none

[a]Substitutions in GyrB are standardized to P0C5C5|1-675,[34] where the QRDR ranges from codon 461 to codon 499. See Table 1 for corresponding sequences.
[b]References where heteropeaks were reported are in bold.
[c]Substitutions GyrA Ala74Ser and Ala90Gly, and GyrB Gly470Ala, Asn499Lys and Δ644–645 were never observed independently.[18,21,26,33,42,46,47]

In several studies, *gyrA* or *gyrB* mutations were revealed as heteropeaks, which are a combination of either wild-type and mutated alleles, or different mutated alleles.[19,21,42,46] Heteropeaks in fluoroquinolone-resistant *M. tuberculosis* have been described in detail previously.[48] In contrast to most bacteria, *M. tuberculosis* acquisition of drug resistance does not occur as a result of horizontal transfer of resistance-bearing genetic elements.[56] Selection of drug resistance during treatment of TB results in a mixture of subpopulations of bacilli, some of which bear different genetic mutations that account for the drug resistance, whereas others are drug susceptible. Consequently, the simultaneous presence of drug-resistant and drug-susceptible bacilli, also designated as heteroresistance, may be found in sputum.[56] These heteroresistant isolates are distinct from isolates with multiple mutations. In fact, fluoroquinolone MICs for isolates with multiple mutations are much higher than those for strains harbouring one mutation, even if presented as a polyclonal population (Table 6). In the presence of anti-TB drugs, drug-resistant bacilli would be expected to outgrow drug-susceptible bacilli over time.

New WHO guidelines include the use of moxifloxacin for the treatment of XDR-TB patients.[57] Although there is cross-resistance within the fluoroquinolone group, moxifloxacin MICs are usually lower than those of other fluoroquinolones (Table 6),[18,20,58] making it a potentially effective drug to treat ofloxacin-resistant TB. The WHO recommendation is supported by a few clinical observations,[59] and also by experiments performed in murine models.[58]

Table 6. Number of *M. tuberculosis* isolates with specific codon mutations and the fluoroquinolone MICs for these isolates

Codon[a]	Number of isolates with this mutation	OFX MIC range (mg/L)	MXF MIC range (mg/L)	Study reference
Single mutations in *gyrA*				
putative promoter	2	1	≤0.125	26
8	1	0.5	≤0.125	26
68	1	<2		24
70	2	1		43[b]
80	7	0.2–>4	≤0.125–1	18, 25, 26, 40[b]
88[a]	11	2–16	2	8, 17, 21,[c] 33,[c] 48,[c] 54, 60,[b] 64
89	2	>2–4	2	26, 48[c]
90[a]	167	0.5–20	0.25–4	3,[b] 6, 8, 9, 10,[c] 11,[c] 12, 16, 17, 18, 19,[c] 20, 21, 23, 24, 26, 27, 31, 33, 42, 43,[b] 46, 47, 48, 53, 54, 55, 61,[b] 62, 63,[b,c] 65, 66[c]
91[a]	44	1–64	1–2	3,[b] 6, 8, 10,[c] 16, 17, 19,[c] 20, 22, 24, 27, 31, 42, 43,[b] 46, 47, 48,[c] 54, 65
92	1	4		11[b]
94[a]	447	0.5–64	0.5–8	3,[b] 6, 8, 9, 10, 12, 16, 17, 19, 20, 21,[c] 22, 23, 24, 25,[c] 26, 27, 31, 33,[c] 38, 39, 41, 42, 43,[b] 46, 47, 48,[c] 53, 54, 55, 61,[b] 62, 63,[b] 65,[c] 66[c]
102	1	ND		20
109	1	2		24
126	4	4.8–8		20, 54
Single mutations in *gyrB*				
446	1	ND		16
447	2	ND		33, 42
461	3	>2–4		8, 33,[c] 42
494	1	>32		7
499	5	1–8	0.25–4	18, 26, 33,[c] 45, 66
500	2	1–>2		33,[c] 43[b]
501	2	12		42
504[a]	3	>2		16, 21[c]
538	1	>2		21[c]
Multiple mutations in *gyrA*				
74+94	21	2–32		46, 47
80+90	7	<0.25–>2	<0.12–0.25	18, 26, 33
80+90+94	1	>2		33[c]
88+90	1	ND		42
88+94	1	>2		9[c]
90+91	11	2–20		17, 46, 47
90+94[a]	48	12–32	>8	19,[c] 21,[c] 23, 26, 33[c] 42,[c] 43,[b] 46, 47
90+102	3	4–4.8		20, 54
91+94	2	>2		17, 19
91+94+94	1	ND		42
94+94[a]	6	>2–12		19,[c] 21,[c] 42
94+94+94[a]	1	>2		21[c]
Multiple mutations in *gyrB*				
461+470[a]	1	ND		42
Multiple mutations in *gyrA* and *gyrB*				
90+461[a]	1	8		42
90+499[a]	1	ND		42
90+94+499[a]	1	ND		42
90+91+94+94+499[a]	1	ND		42
90+500	1	>12		42

Continued

Table 6. *Continued*

Codon[a]	Number of isolates with this mutation	OFX MIC range (mg/L)	MXF MIC range (mg/L)	Study reference
94+461[a]	1	ND		42
94+499[a]	2	>2		21,[c] 42
94+504	1	>2		21[c]
94+Δ644+645	1	4	2	26

CIP, ciprofloxacin; LVX, levofloxacin; MXF, moxifloxacin; ND, not determined; OFX, ofloxacin.
[a]Codon in which heterogeneity was reported.
[b]Studies in which MIC was measured for levofloxacin and/or ciprofloxacin. In Takiff *et al.*,[3] Bozeman *et al.*,[61] Xu *et al.*[63] and Soudani *et al.*,[11] MIC was measured only for ciprofloxacin; in Perlman *et al.*[60] and Yin and Yu,[43] MIC was measured only for levofloxacin.
[c]Studies in which MIC is not specified for every distinct mutation.

Table 7. GyrA and GyrB substitutions demonstrated and not demonstrated to confer fluoroquinolone resistance in *M. tuberculosis*

Gyrase subunit alteration		IC_{50} (mg/L)		
GyrA	GyrB	OFX	MXF	Study reference
WT	WT	2–10	1–2	18, 37
Thr80Ala	WT	5	1	18
Ala90Gly	WT	10	2	18
Thr80Ala+Ala90Gly	WT	2.5	0.5	18
Glu21Gln+Ser95Thr+Gly668Asp+Ala74Ser	WT	16	14	37
Gly88Ala	WT	40	10	64
Gly88Cys	WT	50	35	64
Ala90Val	WT	100	35	18
Asp94Gly	WT	350	50	18
Asp94His	WT	800	90	18
Ala90Val + Asp94Gly	WT	>1600	>160	18
WT	**Asn499Asp**	120	35	18

IC_{50}, 50% inhibitory concentration (measured by inhibition of 50% of DNA supercoiling); MXF, moxifloxacin; OFX, ofloxacin; WT, wild-type.
Substitutions not demonstrated to confer fluoroquinolone resistance in *M. tuberculosis* are in italics.
Substitutions demonstrated to confer fluoroquinolone resistance in *M. tuberculosis* are in bold.

There were some limitations of this review. First, research studies published in languages other than English were not included. Given that some mutations have been reported more frequently in certain populations, we may have excluded mutations associated with fluoroquinolone resistance simply by excluding non-English studies. Second, genotyping lineage information was only available for 10 of the 42 studies reviewed; this information would have been useful in differentiating the polymorphisms predominant in a specific lineage. Third, MEDLINE was the only database searched. Although it contains more than 20 million citations, other databases may contain publications that would have met the inclusion criteria for this study.

This extensive review of mutations described in the *gyrA* and *gyrB* genes in conjunction with the proposed consensus numbering system will facilitate the identification of novel mutations in *M. tuberculosis* isolates. New molecular testing methods may benefit from this information to enhance detection of fluoroquinolone-resistant *M. tuberculosis*.

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Transparency declarations

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Author contributions

F. M., A. W. K. and A. A. performed the literature review. F. M. wrote the first draft of the article. T. R. S. designed the study and provided substantial input in interpreting the data and writing the article. A. B., Y. F. van der H. and C. M. assisted with the literature review and cross-referencing of information. E. C. and A. A. provided substantial input in interpreting the data and writing the article.

Supplementary data

Figures S1, S2 and S3 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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