Frequency and Geographic Distribution of gyrA and gyrB Mutations Associated with Fluoroquinolone Resistance in Clinical Mycobacterium tuberculosis Isolates: A Systematic Review

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Abstract

Background
The detection of mutations in the gyrA and gyrB genes in the Mycobacterium tuberculosis genome that have been demonstrated to confer phenotypic resistance to fluoroquinolones is the most promising technology for rapid diagnosis of fluoroquinolone resistance.

Methods
In order to characterize the diversity and frequency of gyrA and gyrB mutations and to describe the global distribution of these mutations, we conducted a systematic review, from May 1996 to April 2013, of all published studies evaluating Mycobacterium tuberculosis mutations associated with resistance to fluoroquinolones. The overall goal of the study was to determine the potential utility and reliability of these mutations as diagnostic markers to detect phenotypic fluoroquinolone resistance in Mycobacterium tuberculosis and to describe their geographic distribution.

Results
Forty-six studies, covering four continents and 18 countries, provided mutation data for 3,846 unique clinical isolates with phenotypic resistance profiles to fluoroquinolones. The gyrA mutations occurring most frequently in fluoroquinolone-resistant isolates, ranged from 21–32% for D94G and 13–20% for A90V, by drug. Eighty seven percent of all strains that were phenotypically resistant to moxifloxacin and 83% of ofloxacin resistant isolates...
contained mutations in gyrA. Additionally we found that 83% and 80% of moxifloxacin and ofloxacin resistant strains respectively, were observed to have mutations in the gyrA codons interrogated by the existing MTBDRsl line probe assay. In China and Russia, 83% and 84% of fluoroquinolone resistant strains respectively, were observed to have gyrA mutations in the gene regions covered by the MTBDRsl assay.

Conclusions
Molecular diagnostics, specifically the Genotype MTBDRsl assay, focusing on codons 88–94 should have moderate to high sensitivity in most countries. While we did observe geographic differences in the frequencies of single gyrA mutations across countries, molecular diagnostics based on detection of all gyrA mutations demonstrated to confer resistance should have broad and global utility.

Introduction
Mycobacterium tuberculosis (Mtb) is a worldwide public health threat responsible for approximately 8.6 million incident cases of tuberculosis (TB) and an estimated 1.3 million deaths annually [1]. The emergence and increasing prevalence of Mtb strains resistant to first and second line antituberculous medications are exacerbating the global TB epidemic [2]. Multidrug resistant (MDR) strains are Mtb strains resistant to both rifampicin (RIF) and isoniazid (INH), the most effective first-line drugs. Extensively drug resistant (XDR) Mtb strains, are defined as strains with MDR plus resistance to any fluoroquinolone (FQ) and one of the second-line injectable drugs used commonly for treating TB [3]. As of 2012, the World Health Organization (WHO) estimated the global prevalence of MDR-TB to be 3.6% among new TB cases and 20% among recurrent TB cases [1].

As M/XDR-TB rates continue to increase, the development and implementation of rapid diagnostic systems for the detection of microbial resistance to prevent further transmission and promptly implement appropriate drug regimens are needed [4]. Automated liquid culture systems have significantly shortened turnaround times for drug susceptibility tests (DSTs) compared to solid media, but bacteriological assays are technically demanding and still require a sophisticated biosafety environment and approximately 7 to 10 days to complete [4]. Detection of genetic mutations that confer resistance to certain antimicrobial agents represents a more rapid alternative [4]. Currently, the only broadly available commercial assay for the rapid detection of second-line-drug resistance, including FQ resistance, the MTBDRsl assay (Hain Life-science, Nehren, Germany), detects only the most common mutations found in the quinolone resistance determining region (QRDR) of gyrA [5].

The main cellular target of FQs in Mtb is the DNA gyrase, a type II topoisomerase, which consists of two A and two B subunits encoded by gyrA and gyrB genes, respectively [2]. The genetic mechanism of resistance to FQs is a result of changes in the DNA gyrase, particularly, mutations in the QRDR of gyrA (codons 74 to 113) [6] and gyrB (codons 500 to 538) [7]. It has been estimated that roughly 60 to 90% of Mtb clinical isolates with FQ resistance have mutations in the QRDR of gyrA, particularly in codons 88, 90, 91, and 94 [8–10]. Mutations in gyrB have also been associated with FQ resistance, but with lower sensitivity and specificity, and they often co-occur with canonical gyrA mutations [11–15] and most often occur in codons 500 and 538 [16]. While most Mtb strains with gyrA mutations in the QRDR are FQ resistant,
nearly all isolates with a wild type QRDR are FQ susceptible. The exceptions are the polymorphisms of \textit{gyrA} at codons 21, 95 and 668 \cite{14,17,18}, which do not appear to be related to resistance.

FQs have potent \textit{in vitro} activities against \textit{Mtb} \cite{19}. However, FQs are widely used to treat bacterial infections of the respiratory, gastrointestinal, and urinary tract as well as sexually transmitted diseases, further contributing to the increasing levels of FQ resistance in \textit{Mtb} \cite{20,21}. FQs have proven to be among the most effective second-line anti-mycobacterial drugs \cite{14,21} and are recommended for the treatment of drug-resistant TB and for persons intolerant of current first-line therapy \cite{17,22}. While resistance to some of the older generation of FQs has been shown to emerge during treatment of patients infected with FQ-susceptible strains \cite{20}, newer generation FQs have become vital in the successful treatment of drug resistant TB \cite{2,3,23}. As a result of the promising clinical activity of these newer FQs, the WHO currently recommends levofloxacin or moxifloxacin for the treatment of XDR-TB when ofloxacin resistance is present \cite{24,25}.

In order to characterize the \textit{gyrA}\ and \textit{gyrB} mutations associated with global phenotypic resistance to the most commonly used FQs in \textit{Mtb} we conducted a systematic review of English language studies from May 1996 to April 2013. The overall goals of the study were to: 1) characterize the diversity and frequency of \textit{gyrA} and \textit{gyrB} mutations in \textit{Mtb} and 2) to describe the global distribution of these mutations to help determine their potential utility and reliability as diagnostic markers for detecting phenotypic FQ resistance in \textit{Mtb}.

### Methods

#### Literature Search

A Medline search was conducted of all publications investigating \textit{gyrA} and \textit{gyrB} mutations associated with phenotypic FQ resistance in \textit{Mtb}. The search was restricted to studies published from May 1996 through April 15, 2013, including those studies available online prior to publication. MEDLINE/PubMed key search terms used with the help of Boolean operators (‘and’, ‘or’) were: “tuberculosis,” “fluoroquinolone,” “resistance,” “resistant” “\textit{gyrA},” “\textit{gyrB},” “mutation,” “sequence.”

#### Study Selection Criteria

Study selection criteria were similar to those described in Georghiou et al. \cite{26}. Studies were included if they met the following predetermined criteria: i) published in English ii) presented original data and iii) assessed drug resistance mutations in clinical \textit{Mtb} strains resistant to FQs (\textit{in vitro} studies were excluded as laboratory generated mutations have been observed to be different from those found in clinical isolates) \cite{27}. Studies were also excluded if they did not mention the specific FQ tested, did not perform or describe details of phenotypic drug susceptibility testing, did not perform sequencing as a method for determining mutations associated with drug resistance. Additionally, studies were excluded if they did not mention the country the clinical isolates originated from or if they listed multiple countries and did not distinguish clinical isolates by country.

#### Data Extraction and Entry

The following background variables were collected from the selected publications: author(s), year of publication, geographic origin of clinical strains, the reference strain used, methods for testing phenotypic drug susceptibility and genotypic mutations, MIC levels for each drug, genes sequenced, and loci of genes sequenced. The following mutation information was also
recorded: specific gene mutation(s) found, FQ drug utilized for selection, number of resistant and susceptible isolates tested, and number of resistant and susceptible isolates demonstrating a mutation. Data was recorded and compiled using Microsoft Excel (Microsoft, Redmond, WA).

Data Collation and Cumulative Mutation Frequency Calculations

Data concerning mutations associated with FQ resistance were grouped by gene and stratified by the drug resistance phenotype associated with the mutation. Studies that specifically reported multiple mutations within a gene were also analyzed separately in order to determine the frequency of multiple mutations in genes associated with FQ resistance. Each mutation reported in a resistant *Mtb* isolate was considered independent of all others within and between studies (except where otherwise noted for multiple mutations in the same gene) and recorded as one instance of the mutation in the numerator of the cumulative mutation frequency calculations.

Cumulative mutation frequency in resistant isolates was calculated as the number of resistant isolates in which the mutation was found, divided by the total number of phenotypically resistant isolates tested across studies. Cumulative mutation frequency in susceptible isolates was calculated as the number of susceptible isolates in which the mutation was found, divided by the total number of susceptible isolates tested across studies. As not all studies examined all mutations or all genes associated with resistance, isolates from a study were only included in the denominator of a cumulative frequency mutation calculation for a particular mutation if that mutation could have been detected in that study (i.e. the study sequenced the appropriate section of the gene). In order to accurately assess which gene fragments had been sequenced for each isolate, the exact start and end points of the gene fragments sequenced had to be determined. These endpoints were identified by entering the published primer sequences into the NCBI BLAST (Basic Local Alignment Search Tool) with *Mtb* H37Rv complete genome selected as the reference genome, Accession number NC_000962.3 and mapping the coordinates on *Mtb* H37Rv. Sequence fragments were inferred for articles that did not include primer sequences by using the outermost identified mutations as sequence endpoints. If several primers were included and sequenced fragments overlapped, the final dataset included only the outermost/inclusive primers.

The cumulative mutation frequency tables presented in the main body of the review represent the mutations that reached a frequency threshold, described as the following: 1) Isolates were included if a mutation was observed in at least two studies and reported resistance to at least two FQs with a frequency of at least 1% for any one of the FQs tested; 2) Mutations were excluded from the main tables when the frequencies of the mutation were equal in resistant and susceptible strains. Due to the large number of mutations reported (146 total), this frequency threshold was used to report only the most frequently reported mutations in the main tables. All mutations not meeting the above mentioned criteria, are available in S1 Table.

Results

Description of Included Studies

Fig. 1 illustrates the study selection and exclusion process utilized for this review. Initial search parameters identified 193 studies published from May, 1996 through April 15, 2013. Forty-six publications met all eligibility criteria and were included in the review [3, 4, 8–10, 12, 15, 18, 20, 22, 27–62]. (PRISMA checklist included in S2 Table).

Of the 46 studies included, the earliest was published in 1996, with 23 (50%) published in the last three years (Table 1). Altogether, mutation data was provided for 3,846 unique clinical *Mtb* isolates with various phenotypic resistance profiles to FQs. The reported geographic origins of these strains were diverse, covering four continents and 18 countries.
Fig 1. Study Selection Process and Reason for Exclusion of Studies.

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Table 1. Details of Studies Included in Review and Source of *Mycobacterium tuberculosis* Isolates.

| PubMed ID | Author (Year) | # of FLQ Isolates Examined | Origin of Isolates | Molecular Technique | Clinical Institution(s) Providing Isolates | Year of Collection |
|-----------|---------------|-----------------------------|-------------------|-------------------|-------------------------------------------|-------------------|
| 23491718  | Chernyaeva et al. (2013) | 50 | Russia | Sequencing | TB Dispensary | 2011 |
| 23561273  | Jnawali et al. (2013) | 123 | South Korea | PCR & Sequencing | Korea Mycobacterium Resource Center | 2009–2010 |
| 23019190  | Nosova et al. (2013) | 68 | Russia | Sequencing & TB-BIOCHIP-2 | Not Stated | Not Stated |
| 23146281  | Poudel et al. (2013) | 13 | Nepal | PCR & Sequencing | German Nepal Tuberculosis Project | 2007–2010 |
| 22552454  | Chen et al. (2012) | 93 | China | PCR & Sequencing | Not Stated | 2009–2010 |
| 22526012  | Long et al. (2012) | 177 | China | PCR & Sequencing | National Tuberculosis Reference Laboratory | Not Stated |
| 22357804  | Sirgel et al. (2012) | 177 | South Africa | Sequencing | Not Stated | 2007–2009 |
| 22330913  | Streicher et al. (2012) | 181 | South Africa | Sequencing | National Health Laboratory Service | 2006–2008 |
| 22421328  | Suzuki et al. (2012) | 59 | Japan | PCR & Sequencing | 11 Hospitals in Japan | Not Stated |
| 23205246  | Tahmasebi et al. (2012) | 97 | Iran | PCR-SSCP & Sequencing | Mycobacteriology Research Center, Masih Daneshvari Hospital | Not Stated |
| 22553245  | Yuan et al. (2012) | 58 | China | PCR & Sequencing | Jiangxi Chest Hospital | 2010–2011 |
| 22560167  | Zhu et al. (2012) | 227 | China | PCR & Sequencing | Not Stated | 2007–2010 |
| 21911575  | Ali et al. (2011) | 39† | Pakistan | PCR & Sequencing | Aga Khan University Clinical Microbiology Laboratory | 2004–2009 |
| 22152119  | Anand et al. (2011) | 39 | India | Sequencing | Not Stated | Not Stated |
| 2151549   | Ando et al. (2011) | 33 | Japan | PCR, Sequencing & LIPA | Nine Hospitals in Japan | 2002 |
| 21443804  | Cui et al. (2011) | 192 | China | PCR & Sequencing | National Center for Global Health and Medicine | 2003–2008 |
| 21653760  | El Sahly et al. (2011) | 36 | United States | Sequencing | Mycobacteriology Laboratory at Texas Department of State Health Services | 2007–2008 |
| 21562102  | Huang et al. (2011) | 74‡ | Taiwan | GenoType MTBDRsl & PCR | Various Hospitals | 2008–2009 |
| 21450523  | Hu et al. (2011) | 31 | China | Sequencing | Local TB Dispensaries | 2004–2005 |
| 21632897  | Kontsevaya et al. (2011) | 51 | Russia | Sequencing, Pyrosequencing & GenoType MTBDRsl | Various TB Clinics in Samara Region, Russian Federation | 2008 |
| 21555766  | Sekiguchi et al. (2011) | 11 | Japan | PCR | Not Stated | Not Stated |
| 21623040  | Singh et al. (2011) | 8 | India | PCR & Sequencing | Mycobacterial Repository Centre of the Institute | 2004–2008 |
| 22115861  | Zhao et al. (2011) | 125 | China | MAS-PCR, PCR-RFLP & Sequencing | Not Stated | Not Stated |
| 20335420  | Brossier et al. (2010) | 52 | France | Sequencing & GenoType MTBDRsl | French Reference Center for Mycobacteria | 2005–2009 |
| 20573868  | Kiet et al. (2010) | 62 | Vietnam | Sequencing & GenoType MTBDRsl | Pham Ngoc Thach Hospital | 2005–2006 |

(Continued)
A total of 146 unique mutations were reported relative to the reference H37Rv genome: gyrA (76 unique mutations, 34 single mutations and 42 multiple mutations), gyrB (28 unique mutations, 25 single mutations and 3 multiple mutations) and gyrA and gyrB (42 multiple mutations). We evaluated the DST methods and critical drug concentrations used in each study to define whether a strain was phenotypically resistant or not. Table 2 shows the DST methods.

Table 1. (Continued)

| PubMed ID | Author (Year) | # of FLQ Isolates Examined | Origin of Isolates | Molecular Technique | Clinical Institution(s) Providing Isolates | Year of Collection |
|-----------|---------------|----------------------------|-------------------|---------------------|-------------------------------------------|-------------------|
| 20956608  | Lau et al. (2010) | 71 99 | China | PCR & Sequencing | Queen Mary Hospital and Grantham Hospital | 2003–2007 2008–2009 |
| 20452372  | Yin et al. (2010) | 62 | China | PCR & Sequencing | Guangdong Chest Hospital | 2008–2009 |
| 19846642  | Bravo et al. (2009) | 102 | Philippines | PCR & Pyrosequencing | University of the Philippines-Philippine General Hospital | Not Stated |
| 19721073  | Duong et al. (2009) | 109 | Vietnam | Sequencing | Pham Ngoc Thach Hospital | 2005–2007 |
| 19386845  | Hillemann et al. (2009) | 106 | Germany | Sequencing & GenoType MTBDRsl | National Reference Laboratory | Not Stated |
| 20028780  | Perdigao et al. (2009) | 26 | Portugal | PCR & Sequencing | Hospitals and Laboratories in Lisbon’s Health Region | 2005 |
| 19024017  | Antonova et al. (2008) | 107 | Russia | PCR, Biochip & Sequencing | Not Stated | Not Stated |
| 18559646  | Mokrousov et al. (2008) | 71 | Russia | PCR & Sequencing | St. Petersburg Research Institute of Phthisiopulmonology | 2006 |
| 18164184  | Sun et al. (2008) | 110 | China | PCR, DHPLC & Sequencing | Beijing Chest Hospital | 2002–2004 |
| 18544197  | van Doorn et al. (2008) | 82 | Vietnam | PCR, RT-PCR & Sequencing | Pham Ngoc Thach Hospital | 2005–2006 |
| 17360809  | Chan et al. (2007) | 250 | China | PCR-SSCP/ MPAC & Sequencing | Grantham Hospital and Public Health Laboratory | 1994–2004 |
| 17934259  | Escribino et al. (2007) | 18 | Spain | PCR & Sequencing | Not Stated | Not Stated |
| 17434825  | Sulochana et al. (2007) | 118 | India | PCR & Sequencing | Not Stated | Not Stated |
| 17412727  | Wang et al. (2007) | 42 | Taiwan | PCR & Sequencing | Tertiary Care Referral Centre | 2004–2005 |
| 16584301  | Kam at al. (2006) | 143 | China | Sequencing | TB Reference Laboratory, Department of Health | 1999–2003 |
| 16204341  | Huang et al. (2005) | 141 | Taiwan | PCR & Sequencing | Kaohsiung Veterans General Hospital | 1995–2003 |
| 15195248  | Post et al. (2004) | 13 | South Africa | Sequencing | Not Stated | Not Stated |
| 12044302  | Lee et al. (2002) | 100 | Singapore | PCR & Sequencing | Central Tuberculosis Laboratory | Not Stated |
| 11796356  | Siddiqi et al. (2002) | 68 | India | PCR & Sequencing | Outpatient hospitals and National Mycobacterial Repository | 1995–1998 |
| 8737156   | Williams et al. (1996) | 9 | China | PCR & Sequencing | Not Stated | Not Stated |
| 8896523   | Xu et al. (1996) | 19 | United States | PCR & Sequencing | Public Health Research Institute Tuberculosis Center | Not Stated |

*Does not include reference strain
†Included S95T; not reported here
‡Examined 234 isolates, reported 74
Table 2. Drug Susceptibility Testing (DST) Methods Employed in Publications.

| Author          | DST Method | Second Generation |            | Third Generation | Fourth Generation |
|-----------------|------------|------------------|------------|------------------|-------------------|
|                 |            | CIPRO | OFL | LEVO | SPAR | GAT | MOX | SITA |
| Tahmasebi [52]  | LJ         | 2.0* | —   | —    | —    | —   | —   | —    |
| et al.          |            |      |      |      |      |      |      |      |
| Wang [20] et al.| LJ         | 2.0* | 2.0*| 1.0‡‡| —    | —    | 0.5‡‡| —    |
| Hu [46] et al.  | LJ         | 2.0* | 2.0*| 1.0‡‡| —    | —    | —    | —    |
| Chen [53] et al.| LJ         | 1.0–16.0††| 2.0–16.0† | —    | 0.125–8.0‡‡| 0.125–16.0‡‡| —    |
| Poudel [54] et al.| LJ         | —    | 2.0*| —    | —    | —    | —    | —    |
| Yuan [55] et al.| LJ         | —    | 2.0*| —    | —    | —    | —    | —    |
| Williams [31]   | LJ         | —    | 2.0*| —    | —    | —    | —    | —    |
| Jnawali [56] et al.| LJ         | —    | 2.0*| —    | —    | —    | —    | —    |
| Zhao [41] et al.| LJ         | —    | 2.0*| —    | —    | —    | —    | —    |
| Brossier [47] et al.| LJ         | —    | 2.0*| —    | —    | —    | —    | —    |
| Kiet [40] et al.| LJ         | —    | 2.0*| —    | —    | —    | —    | —    |
| Duong [12] et al.| LJ         | —    | 2.0*| —    | —    | —    | —    | —    |
| Mokrousov [8] et al.| LJ         | —    | 2.0*| —    | —    | —    | —    | —    |
| van Doorn [39] et al.| LJ         | —    | 2.0*| —    | —    | —    | —    | —    |
| Hillemann [9] et al.| LJ/MGIT 960| —    | 2.0*| —    | —    | —    | —    | —    |
| Nosova [57] et al.| LJ         | —    | 2.0*| 2.0‡‡| —    | —    | 0.5‡‡| —    |
| Anand [22] et al.| LJ         | 2.0–4.0† | 2.0‡‡| —    | —    | 2.0–5.0‡‡| —    |
| Chemyaeva [51] et al.| LJ         | 2.0–10.0* | —    | —    | —    | —    | —    |
| Antonova [29] et al.| LJ         | 2.0*, 10.0† | —    | —    | —    | —    | —    |
| Long [62] et al.| LJ         | 5.0–50.0† | 2.0–20.0‡‡| —    | —    | —    | —    |
| Kam [49] et al.| LJ/MGIT 960| —    | 0.5*, 1.0†, 2.0*, 4.0†, 8.0†, 16.0† | —    | —    | 0.5*, 1.0‡‡, 2.0‡‡, 4.0‡‡, 8.00‡‡, 16.00‡‡| —    |
| Sun [27] et al.| LJ         | —    | 0.5*, 1.0†, 2.0*, 4.0†, 8.0†, 10.0†, 16.0†, 20.0† | —    | —    | —    | —    |
| Sulochana [38] et al.| LJ         | 8.0† | —    | —    | —    | —    | —    |
| Chan [36] et al.| LJ         | —    | —    | —    | —    | 4.8‡‡| —    |
| Siddiqi [33] et al.| LJ         | —    | —    | —    | —    | 2.0‡‡| —    |
| Perdigao [48] et al. 2007| BACTEC 460| —    | 2.0*| —    | —    | —    | —    |
| Zhu [58] et al.| MGIT 960  | —    | 2.0*| —    | —    | —    | —    |
| Kontsevaya [4] et al.| MGIT 960  | —    | 2.0*| —    | —    | 2.0†| —    |
| Streicher [59] et al.| MGIT 960  | —    | 2.0*| —    | —    | —    | —    |
| Cui [15] et al.| MGIT 960  | —    | 2.0*| —    | —    | —    | —    |
| Sigel [60] et al.| MGIT 960  | 0.5–10.0††| —    | —    | —    | 0.125–2.0††| —    |

(Continued)
and critical concentrations used in each of the included studies and whether or not they con-
formed to published reference standards. The drug concentrations used in 35 of the 46 (76%) 
studies conformed to at least one national or international published standard, 4 (9%) studies 
were conducted in national reference laboratories. The remaining 7 (15%) studies did not doc-
ument a specific reference laboratory standard.

**gyrA Mutations Associated with Fluoroquinolone Resistance**

Of the 46 papers examined in this review, all 46 studied resistance-associated markers within 
**gyrA.** Fig. 2 shows the **gyrA** studies as a heat map of the number of isolates evaluated in all 46 
studies as well as the locations of the mutations found in **gyrA.** Thirty-four studies sequenced 
the QRDR of the **gyrA** gene, 11 studies sequenced part of the QRDR of the **gyrA** gene; only one 
study sequenced the entire **gyrA** gene.

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**Table 2. (Continued)**

| Author          | DST Method          | Second Generation | Third Generation | Fourth Generation |
|-----------------|---------------------|-------------------|------------------|-------------------|
|                 |                     | CIPRO | OFL   | LEVO | SPAR | GAT  | MOX  | SITA |
| Singh [44] et al. | Middlebrook 7H9     | —     | 8.0†  | 16.0† | —    | —    | —    | —    |
| Sekiguchi [50] et al. | Middlebrook 7H10   | 0.5‡  | —     | 0.5‡  | —    | 0.06† | —    | —    |
| Xu [32] et al.   | Middlebrook 7H10    | 2.0*  | —     | —    | —    | —    | —    | —    |
| Ali [42] et al.  | Middlebrook 7H11    | 2.0*  | —     | —    | —    | —    | —    | —    |
| Huang [45] et al. | Middlebrook 7H11    | 2.0*  | 2.0*  | 1.0‡‡| —    | —    | —    | —    |
| Suzuki [51] et al. | Middlebrook 7H11   | 6.25–50.0†| — | 3.13–25.0†| 1.56–12.5‡| 0.78–6.25†| 0.78–12.5‡| 0.39–12.5‡|
| Escribano [37] et al. | Middlebrook 7H11 | 16.0‡  | 16.0† | 8.0‡‡| —    | 2.0‡‡| 4.0‡‡| —    |
| Bravo [10] et al. | Middlebrook 7H10    | —     | 2.0*  | —    | —    | —    | —    | —    |
| Lau [18] et al.  | Middlebrook 7H10    | —     | 2.0*  | —    | —    | —    | 1.0‡‡| —    |
| Post [34] et al. | Middlebrook 7H10    | —     | 2.0*  | —    | —    | —    | —    | —    |
| Huang [35] et al. | Middlebrook 7H11    | —     | 2.0*  | —    | —    | —    | —    | —    |
| Yin [28] et al.  | Middlebrook 7H11    | —     | —     | 1.0, 10.0‡‡| —    | —    | —    | —    |
| El Sahly [43] et al. | Agar proportion indirect susceptibility assay | — | — | — | — | 0.5‡‡| — |
| Ando [3] et al.  | Broth MIC; Egg based Ogawa medium | 2.0–16.0‡‡| — | 2.0–16.0‡‡| 1.0–8.0‡‡| — | — | — |
| Lee [30] et al.  | E-test              | —     | —     | —    | —    | 32.0‡‡| —    | —    |

CIPRO = Ciprofloxacin, GAT = Gatifloxacin, LEVO = Levofloxacin, MOX = Moxifloxacin, OFL = Ofloxacin, SITA = Sitafloxacin, SPX = Sparfloxacin, NM = MIC not mentioned, LJ = Lowenstein-Jensen
— = Indicates fluoroquinolone not tested in this study
†DST conforms to published standard
‡DST above published standard
††DST below published standard
**Absolute concentration, not yet validated
†††DST range above and below published standard
‡‡‡No published standard
*In gyrB only

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Table 3 shows the cumulative frequencies of the most commonly reported mutations in the \textit{gyrA} gene associated with resistance to the primary FQs across publications. Resistance to ofloxacin, levofloxacin and moxifloxacin was studied in the largest number of isolates, but it is important to note that the primary canonical mutations listed in Table 3 appeared to be associated with resistance to all of the clinically relevant FQs, suggesting some level of cross-resistance is likely. Additionally, a subset of studies specifically examined and found evidence of cross resistance associated with these mutations, for example, most moxifloxacin resistant isolates with the A90V mutation (18%) were also resistant to ofloxacin (17%).

Eighty seven percent of the moxifloxacin resistant isolates and 83% of the ofloxacin resistant isolates had mutations in their \textit{gyrA} genes, with most mutations occurring in codons 88–94 (Table 3, S1 Table and S3 Table). The cumulative frequency of individual mutations associated with FQ resistance was highest for the \textit{gyrA} mutation D94G, ranging from 21–32% in FQ-resistant isolates depending on the specific FQ tested. The \textit{gyrA} A90V mutation was the second most frequent mutation observed in FQ resistant isolates, and was found in 13–20% of FQ-resistant isolates depending on the FQ tested. Across all drugs tested, the \textit{gyrA} mutations G88C and D94V were least frequent (1–2%).

Most importantly, none of the mutations listed in Table 3 occurred in more than a few of the many thousands of FQ susceptible isolates evaluated. Of the 41 studies reporting single
Table 3. Cumulative Frequencies of the Most Frequently Occurring Mutations within *gyrA* Gene among *Mycobacterium tuberculosis* Isolates Resistant to Fluoroquinolones. Mutations are listed in order of descending frequency.

| Codon | Substitution | FLQ Tested | # Resistant Isolates Examined | # Susceptible Isolates Examined | # Resistant Isolates with Mutation | # Susceptible Isolates with Mutation | Frequency of Mutation among Resistant Isolates | Frequency of Mutation among Susceptible Isolates |
|-------|--------------|------------|-------------------------------|---------------------------------|-----------------------------------|--------------------------------------|-----------------------------------------------|-----------------------------------------------|
| 94    | Asp→Gly     | OFL 1995   | 1572                          | 566                             | 0                                 | 0.28                                 | 0                                             |                                               |
|       |              | MOX 357    | 540                           | 114                             | 0                                 | 0.32                                 | 0                                             |                                               |
|       |              | LEVO 412   | 248                           | 105                             | 0                                 | 0.25                                 | 0                                             |                                               |
|       |              | CIPRO 334  | 287                           | 81                              | 0                                 | 0.24                                 | 0                                             |                                               |
|       |              | GAT 198    | 91                            | 56                              | 0                                 | 0.28                                 | 0                                             |                                               |
|       |              | SPX 109    | 0                             | 23                              | 0                                 | 0.21                                 | NA                                            |                                               |
|       |              | SITA 59    | 0                             | 15                              | 0                                 | 0.25                                 | NA                                            |                                               |
|       | Asp→Ala     | OFL 1995   | 1572                          | 177                             | 1                                 | 0.09                                 | 0                                             |                                               |
|       |              | MOX 357    | 540                           | 43                              | 0                                 | 0.12                                 | 0                                             |                                               |
|       |              | LEVO 412   | 248                           | 46                              | 0                                 | 0.11                                 | 0                                             |                                               |
|       |              | CIPRO 334  | 287                           | 36                              | 0                                 | 0.11                                 | 0                                             |                                               |
|       |              | GAT 198    | 91                            | 26                              | 0                                 | 0.13                                 | 0                                             |                                               |
|       |              | SPX 109    | 0                             | 19                              | 0                                 | 0.17                                 | NA                                            |                                               |
|       |              | SITA 59    | 0                             | 10                              | 0                                 | 0.17                                 | NA                                            |                                               |
|       | Asp→Asn     | OFL 1995   | 1572                          | 122                             | 1                                 | 0.06                                 | 0                                             |                                               |
|       |              | MOX 357    | 540                           | 22                              | 1                                 | 0.06                                 | 0                                             |                                               |
|       |              | LEVO 412   | 248                           | 22                              | 0                                 | 0.05                                 | 0                                             |                                               |
|       |              | CIPRO 334  | 287                           | 28                              | 1                                 | 0.08                                 | 0                                             |                                               |
|       |              | GAT 198    | 91                            | 13                              | 1                                 | 0.07                                 | 0.01                                          |                                               |
|       |              | SPX 109    | 0                             | 5                               | 0                                 | 0.05                                 | NA                                            |                                               |
|       |              | SITA 59    | 0                             | 5                               | 0                                 | 0.08                                 | NA                                            |                                               |
|       | Asp→Tyr     | OFL 1995   | 1572                          | 79                              | 0                                 | 0.04                                 | 0                                             |                                               |
|       |              | MOX 357    | 540                           | 14                              | 0                                 | 0.04                                 | 0                                             |                                               |
|       |              | LEVO 412   | 248                           | 11                              | 0                                 | 0.03                                 | 0                                             |                                               |
|       |              | CIPRO 334  | 287                           | 19                              | 0                                 | 0.06                                 | 0                                             |                                               |
|       |              | GAT 198    | 91                            | 11                              | 0                                 | 0.06                                 | 0                                             |                                               |
|       |              | SPX 109    | 0                             | 6                               | 0                                 | 0.06                                 | NA                                            |                                               |
|       |              | SITA 59    | 0                             | 5                               | 0                                 | 0.08                                 | NA                                            |                                               |
|       | Asp→His     | OFL 1995   | 1572                          | 21                              | 0                                 | 0.01                                 | 0                                             |                                               |
|       |              | MOX 357    | 540                           | 4                               | 0                                 | 0.01                                 | 0                                             |                                               |
|       |              | LEVO 412   | 248                           | 3                               | 0                                 | 0.01                                 | 0                                             |                                               |
|       |              | CIPRO 334  | 287                           | 1                               | 0                                 | 0                                   | 0                                             |                                               |
|       |              | GAT 198    | 91                            | 1                               | 0                                 | 0.01                                 | 0                                             |                                               |
|       | Asp→Val     | OFL 1995   | 1572                          | 40                              | 0                                 | 0.04                                 | 0                                             |                                               |
|       |              | MOX 357    | 540                           | 5                               | 0                                 | 0.01                                 | 0                                             |                                               |
|       |              | LEVO 412   | 248                           | 4                               | 0                                 | 0.01                                 | 0                                             |                                               |
|       |              | CIPRO 334  | 287                           | 2                               | 0                                 | 0.01                                 | 0                                             |                                               |
|       |              | GAT 198    | 91                            | 2                               | 0                                 | 0.01                                 | 0                                             |                                               |
|       |              | SPX 109    | 0                             | 1                               | 0                                 | 0.01                                 | NA                                            |                                               |
|       |              | SITA 59    | 0                             | 1                               | 0                                 | 0.02                                 | NA                                            |                                               |
|       | Ala→Val     | OFL 1995   | 1572                          | 330                             | 4                                 | 0.17                                 | 0                                             |                                               |
|       |              | MOX 357    | 540                           | 65                              | 0                                 | 0.18                                 | 0                                             |                                               |
|       |              | LEVO 412   | 248                           | 82                              | 0                                 | 0.2                                  | 0                                             |                                               |
|       |              | CIPRO 334  | 287                           | 45                              | 0                                 | 0.13                                 | 0                                             |                                               |
|       |              | GAT 198    | 91                            | 36                              | 0                                 | 0.18                                 | 0                                             |                                               |
|       |              | SPX 109    | 0                             | 16                              | 0                                 | 0.15                                 | NA                                            |                                               |
|       |              | SITA 59    | 0                             | 12                              | 0                                 | 0.2                                  | NA                                            |                                               |

(Continued)
A90V mutations, only two studies (n = 4) reported the A90V mutation in FQ susceptible isolates. Two other mutations were reported in susceptible isolates: D94A and D94N, but less than 1% of susceptible isolates contained these mutations, leaving open the possibility these were likely phenotypic DST errors.

**gyrB Mutations Associated with Fluoroquinolone Resistance**

Eighteen of the 46 (39%) publications included sequence data for **gyrB**. However, overall the **gyrB** mutations have only been evaluated in a few hundred FQ-resistant strains. Mutations of the **gyrB** gene occurred most frequently within ofloxacin resistant isolates (Table 4). The **gyrB** N538D mutation (also reported as N510D in some publications depending on the numbering system used), as well as D500H, T539N and A543V were reported to be rare among ofloxacin-resistant isolates, at frequencies of less than 1%. While the number of susceptible isolates examined for **gyrB** mutations was low, it is important to note than none of them contained mutations listed in Table 4.

**Double Mutations in gyrA Associated with Fluoroquinolone Resistance**

Several studies reported double mutations in **gyrA**, **gyrB** or both **gyrA** and **gyrB**; S3 Table includes double mutations reported within the **gyrA** gene. The most commonly reported double mutations largely included the previously examined A90V mutation. While the cumulative

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**Table 3.** (Continued)

| Codon | Substitution | FLQ Tested | # Resistant Isolates Examined | # Susceptible Isolates Examined | # Resistant Isolates with Mutation | # Susceptible Isolates with Mutation |
|-------|--------------|------------|------------------------------|---------------------------------|-----------------------------------|-------------------------------------|
| 91    | Ser—Pro      | OFL 1995   | 1572                         | 84                              | 0.04                              | 0                                   |
|       |              | MOX 357    | 540                          | 14                              | 0.04                              | 0                                   |
|       |              | LEVO 412   | 248                          | 9                               | 0.02                              | 0                                   |
|       |              | CIPRO 334  | 287                          | 18                              | 0.05                              | 0                                   |
|       |              | GAT 198    | 91                           | 7                               | 0.04                              | 0                                   |
|       |              | SPX 109    | 0                            | 4                               | 0.04                              | NA                                  |
|       |              | SITA 59    | 0                            | 4                               | 0.07                              | NA                                  |
| 88    | Gly—Cys      | OFL 1982   | 1504                         | 17                              | 0.01                              | 0                                   |
|       |              | MOX 357    | 540                          | 5                               | 0.01                              | 0                                   |
|       |              | LEVO 412   | 248                          | 2                               | 0                                  | 0                                   |
|       |              | CIPRO 295  | 287                          | 1                               | 0                                  | 0                                   |
|       |              | GAT 198    | 91                           | 2                               | 0.01                              | 0                                   |
|       |              | SPX 109    | 0                            | 1                               | 0.01                              | NA                                  |
|       |              | SITA 59    | 0                            | 1                               | 0.02                              | NA                                  |
| 126   | Ala—Arg      | OFL 1676   | 1283                         | 4                               | 0                                  | 0                                   |
|       |              | MOX 335    | 523                          | 2                               | 0                                  | 0.01                               |

CIPRO = Ciprofloxacin, GAT = Gatifloxacin, LEVO = Levofoxacin, MOX = Moxifloxacin, OFL = Ofloxacin, SITA = Sitafloxacin, SPX = Sparfloxacin, NA = Not Applicable

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frequencies of gyrA double mutations ranged from 1–3% among resistant isolates, no susceptible isolates were reported to contain any of the double mutations, suggesting that although rare, double gyrA mutations are highly specific predictors of FQ-resistance.

Mutations in gyrA Associated with Fluoroquinolone Resistance by Country

Table 5 shows the cumulative frequencies of gyrA point mutations in FQ resistant isolates by country. The greatest number of studies came from China (n = 13), followed by Russia (n = 5), with all other countries contributing less than four studies each. Both China and Russia reported the gyrB mutation D500H in FQ resistant isolates. In China, 85% of mutations reported were found in codons 88–94, whereas 89% of mutations in Russia were in these codons (the remainder of the mutations occurred outside of these codons and in gyrB). Of the 18 country-specific studies included in our review, 14 reported mutations in codon 90 (all in A90V) with frequencies ranging from 6% of FQ resistant strains in Iran to 30% of FQ resistant strains in the Philippines. Sixteen countries reported mutations in codon 94. For gyrA D94G, the cumulative frequency of the mutation in all FQ resistant strains ranged from 6% in Iran to 56% in South Korea. While A90V and D94G were the most frequently reported mutations overall, four countries reported mutations other than these mutations with higher frequency. In India, the most commonly reported mutation was D94A (20%); in Iran the most commonly reported mutation was D94N (11%); in Portugal the most commonly reported mutation was S91P (42%) and in Spain the most commonly reported mutation was D84G (17%).

Discussion

From the literature reviewed, it is evident that the QRDR of gyrA has been widely studied in FQ resistant Mtb isolates; while the remainder of the gyrA gene and the gyrB gene have been only rarely evaluated. In this review, we found that mutations occurring in the QRDR,
Table 5. Cumulative Frequencies of Selected Mutations within gyrA Gene among *Mycobacterium tuberculosis* Isolates by Country.

| Country | Mutation | # Resistant Isolates Examined | # Susceptible Isolates Examined | # Resistant Isolates with Mutation | # Susceptible Isolates with Mutation | Frequency of Mutation among Resistant Isolates | Frequency of Mutation among Susceptible Isolates |
|---------|----------|------------------------------|--------------------------------|----------------------------------|--------------------------------------|---------------------------------------------|---------------------------------------------|
| China (n = 13) | A90V | 1391 | 1088 | 253 | 0 | 0.18 | 0.00 |
| | D94G | 1391 | 1088 | 394 | 0 | 0.28 | 0.00 |
| | D94A | 1391 | 1088 | 111 | 1 | 0.08 | 0.00 |
| | D94N | 1391 | 1088 | 117 | 4 | 0.08 | 0.00 |
| | S91P | 1391 | 1088 | 51 | 0 | 0.04 | 0.00 |
| | D94Y | 1391 | 1088 | 63 | 0 | 0.05 | 0.00 |
| | D94H | 1391 | 1088 | 18 | 0 | 0.01 | 0.00 |
| | G88C | 1391 | 1088 | 3 | 0 | 0.00 | 0.00 |
| | D500H | 674 | 220 | 3 | 0 | 0.00 | 0.00 |
| France (n = 1) | A90V | 24 | 28 | 4 | 0 | 0.17 | 0.00 |
| | D94G | 24 | 28 | 6 | 0 | 0.25 | 0.00 |
| | D94A | 24 | 28 | 2 | 0 | 0.08 | 0.00 |
| | D94N | 24 | 28 | 2 | 0 | 0.08 | 0.00 |
| | D94H | 24 | 28 | 1 | 0 | 0.04 | 0.00 |
| | G88C | 24 | 28 | 1 | 0 | 0.04 | 0.00 |
| | N538D | 24 | 28 | 1 | 0 | 0.04 | 0.00 |
| Germany (n = 1) | A90V | 32 | 74 | 4 | 0 | 0.13 | 0.00 |
| | D94G | 32 | 74 | 13 | 0 | 0.41 | 0.00 |
| | D94A | 32 | 74 | 5 | 0 | 0.16 | 0.00 |
| | D94N | 32 | 74 | 1 | 0 | 0.03 | 0.00 |
| | S91P | 32 | 74 | 1 | 0 | 0.03 | 0.00 |
| India (n = 4) | A90V | 153 | 158 | 15 | 0 | 0.10 | 0.00 |
| | D94G | 153 | 158 | 14 | 0 | 0.09 | 0.00 |
| | D94A | 153 | 158 | 31 | 0 | 0.20 | 0.00 |
| | D94N | 153 | 158 | 4 | 0 | 0.03 | 0.00 |
| | S91P | 153 | 158 | 2 | 0 | 0.01 | 0.00 |
| | D94Y | 153 | 158 | 2 | 0 | 0.01 | 0.00 |
| | A90V | 18 | 79 | 1 | 0 | 0.06 | 0.00 |
| | D94G | 18 | 79 | 1 | 0 | 0.06 | 0.00 |
| | D94N | 18 | 79 | 2 | 0 | 0.11 | 0.00 |
| Japan (n = 3) | A90V | 537 | 0 | 93 | 0 | 0.17 | NA |
| | D94G | 537 | 0 | 120 | 0 | 0.22 | NA |
| | D94A | 537 | 0 | 90 | 0 | 0.17 | NA |
| | D94N | 537 | 0 | 33 | 0 | 0.06 | NA |
| | S91P | 537 | 0 | 24 | 0 | 0.04 | NA |
| | D94Y | 537 | 0 | 36 | 0 | 0.07 | NA |
| | G88C | 537 | 0 | 6 | 0 | 0.01 | NA |
| Nepal (n = 1) | D94G | 13 | 0 | 7 | 0 | 0.54 | NA |
| | D94A | 13 | 0 | 2 | 0 | 0.15 | NA |
| | D94N | 13 | 0 | 1 | 0 | 0.08 | NA |
| | S91P | 13 | 0 | 1 | 0 | 0.08 | NA |
| | D94Y | 13 | 0 | 1 | 0 | 0.08 | NA |
| | D94H | 13 | 0 | 1 | 0 | 0.08 | NA |
| | A90V | 39 | 0 | 9 | 0 | 0.23 | NA |
| | D94G | 39 | 0 | 14 | 0 | 0.36 | NA |
| | D94A | 39 | 0 | 2 | 0 | 0.05 | NA |
| | D94N | 39 | 0 | 2 | 0 | 0.05 | NA |
| | S91P | 39 | 0 | 1 | 0 | 0.03 | NA |
| | D94Y | 39 | 0 | 5 | 0 | 0.13 | NA |

(Continued)
| Country      | Mutation | # Resistant Isolates Examined | # Susceptible Isolates Examined | # Resistant Isolates with Mutation | # Susceptible Isolates with Mutation | Frequency of Mutation among Resistant Isolates | Frequency of Mutation among Susceptible Isolates |
|-------------|---------|-------------------------------|-------------------------------|-----------------------------------|--------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Philippines (n = 1) | A90V   | 10                            | 92                            | 3                                | 0                                    | 0.30                                          | 0.00                                          |
|              | D94G   | 10                            | 92                            | 3                                | 0                                    | 0.30                                          | 0.00                                          |
|              | D94N   | 52                            | 0                             | 12                               | 0                                    | 0.23                                          | NA                                           |
|              | D94A   | 52                            | 0                             | 16                               | 0                                    | 0.31                                          | NA                                           |
|              | S91P   | 52                            | 0                             | 22                               | 0                                    | 0.42                                          | NA                                           |
| Portugal (n = 1) |         |                               |                               |                                   |                                       |                                               |                                               |
|              | A90V   | 364                           | 238                           | 67                               | 3                                    | 0.18                                          | 0.01                                          |
|              | D94G   | 364                           | 238                           | 122                              | 0                                    | 0.34                                          | 0.00                                          |
|              | D94A   | 364                           | 238                           | 42                               | 0                                    | 0.12                                          | 0.00                                          |
|              | D94N   | 364                           | 238                           | 14                               | 0                                    | 0.04                                          | 0.00                                          |
|              | S91P   | 364                           | 238                           | 10                               | 0                                    | 0.03                                          | 0.00                                          |
|              | D94Y   | 364                           | 238                           | 18                               | 0                                    | 0.05                                          | 0.00                                          |
|              | D94H   | 364                           | 238                           | 5                                | 9                                    | 0.01                                          | 0.04                                          |
|              | G88C   | 364                           | 238                           | 10                               | 0                                    | 0.03                                          | 0.00                                          |
|              | D500H  | 250                           | 143                           | 4                                | 0                                    | 0.02                                          | 0.00                                          |
|              | N538D  | 250                           | 143                           | 4                                | 0                                    | 0.02                                          | 0.00                                          |
| Russia (n = 5) |         |                               |                               |                                   |                                       |                                               |                                               |
|              | A90V   | 364                           | 238                           | 67                               | 3                                    | 0.18                                          | 0.01                                          |
|              | D94G   | 364                           | 238                           | 122                              | 0                                    | 0.34                                          | 0.00                                          |
|              | D94A   | 364                           | 238                           | 42                               | 0                                    | 0.12                                          | 0.00                                          |
|              | D94N   | 364                           | 238                           | 14                               | 0                                    | 0.04                                          | 0.00                                          |
|              | S91P   | 364                           | 238                           | 10                               | 0                                    | 0.03                                          | 0.00                                          |
|              | D94Y   | 364                           | 238                           | 18                               | 0                                    | 0.05                                          | 0.00                                          |
|              | D94H   | 364                           | 238                           | 5                                | 9                                    | 0.01                                          | 0.04                                          |
|              | G88C   | 364                           | 238                           | 10                               | 0                                    | 0.03                                          | 0.00                                          |
|              | D500H  | 250                           | 143                           | 4                                | 0                                    | 0.02                                          | 0.00                                          |
|              | N538D  | 250                           | 143                           | 4                                | 0                                    | 0.02                                          | 0.00                                          |
| Singapore (n = 1) |         |                               |                               |                                   |                                       |                                               |                                               |
|              | A90V   | 48                            | 24                            | 1                                | 0                                    | 0.02                                          | 0.00                                          |
| South Africa (n = 3) |         |                               |                               |                                   |                                       |                                               |                                               |
|              | A90V   | 280                           | 258                           | 65                               | 0                                    | 0.23                                          | 0.00                                          |
|              | D94G   | 280                           | 258                           | 92                               | 0                                    | 0.33                                          | 0.00                                          |
|              | D94A   | 280                           | 258                           | 30                               | 0                                    | 0.11                                          | 0.00                                          |
|              | D94N   | 280                           | 258                           | 27                               | 0                                    | 0.10                                          | 0.00                                          |
|              | S91P   | 280                           | 258                           | 15                               | 0                                    | 0.05                                          | 0.00                                          |
|              | D94Y   | 280                           | 258                           | 2                                | 0                                    | 0.01                                          | 0.00                                          |
|              | G88C   | 275                           | 250                           | 3                                | 0                                    | 0.01                                          | 0.00                                          |
| South Korea (n = 1) |         |                               |                               |                                   |                                       |                                               |                                               |
|              | A90V   | 108                           | 15                            | 16                               | 0                                    | 0.15                                          | 0.00                                          |
|              | D94G   | 108                           | 15                            | 60                               | 0                                    | 0.56                                          | 0.00                                          |
|              | D94A   | 108                           | 15                            | 2                                | 0                                    | 0.02                                          | 0.00                                          |
|              | D94N   | 108                           | 15                            | 3                                | 0                                    | 0.03                                          | 0.00                                          |
|              | S91P   | 108                           | 15                            | 9                                | 0                                    | 0.08                                          | 0.00                                          |
|              | D94Y   | 108                           | 15                            | 2                                | 0                                    | 0.02                                          | 0.00                                          |
|              | D94H   | 108                           | 15                            | 2                                | 0                                    | 0.02                                          | 0.00                                          |
| Spain (n = 1) |         |                               |                               |                                   |                                       |                                               |                                               |
|              | D84G   | 35                            | 60                            | 5                                | 0                                    | 0.14                                          | 0.00                                          |
| Taiwan (n = 3) |         |                               |                               |                                   |                                       |                                               |                                               |
|              | A90V   | 145                           | 520                           | 15                               | 0                                    | 0.10                                          | 0.00                                          |
|              | D94G   | 145                           | 520                           | 51                               | 0                                    | 0.35                                          | 0.00                                          |
|              | D94A   | 145                           | 520                           | 3                                | 0                                    | 0.02                                          | 0.00                                          |
|              | D94N   | 145                           | 520                           | 5                                | 0                                    | 0.03                                          | 0.00                                          |
|              | S91P   | 145                           | 520                           | 2                                | 0                                    | 0.01                                          | 0.00                                          |
|              | D94Y   | 145                           | 520                           | 6                                | 0                                    | 0.04                                          | 0.00                                          |
|              | G88C   | 145                           | 520                           | 6                                | 0                                    | 0.04                                          | 0.00                                          |
| United States (n = 2) |         |                               |                               |                                   |                                       |                                               |                                               |
|              | A90V   | 23                            | 26                            | 4                                | 0                                    | 0.17                                          | 0.00                                          |
|              | D94G   | 23                            | 26                            | 3                                | 0                                    | 0.13                                          | 0.00                                          |
|              | D94A   | 23                            | 26                            | 1                                | 0                                    | 0.04                                          | 0.00                                          |
|              | D94N   | 23                            | 26                            | 3                                | 0                                    | 0.13                                          | 0.00                                          |
|              | D94Y   | 23                            | 26                            | 3                                | 0                                    | 0.13                                          | 0.00                                          |
|              | D94H   | 23                            | 26                            | 2                                | 0                                    | 0.09                                          | 0.00                                          |

(Continued)
specifically in codons 88–94, were found in 85% and 82% of phenotypic moxifloxacin and ofloxacin resistant strains, respectively. These results suggest that \textit{gyrA} mutations in codons 88–94 are likely to be very sensitive markers of phenotypic resistance to FQ drugs in \textit{Mtb} isolates, with high likelihood of cross-resistance to all the major FQs. Only one study included in the review sequenced the entire \textit{gyrA} gene, explaining why very few mutations were reported outside of the QRDR region. The understudied \textit{gyrA} regions may contain mutations that help explain the 15–18% of reported FQ resistant strains that did not appear to have mutations in codons 88–94 of the QRDR of \textit{gyrA}. Additionally the 15–18% of FQ resistant \textit{Mtb} strains with no identified mutation may possess an alternate mechanism of resistance [35, 63, 64]. Low cell wall permeability, efflux-related mechanisms, and drug sequestration or inactivation have been proposed to account for FQ resistance in these isolates [27, 64]. Equally important to the high frequency of the \textit{gyrA} mutations in FQ resistant isolates, is the fact that these mutations occurred in only a few (<1%) FQ susceptible isolates, suggesting these rare mutations are highly specific markers of FQ-resistance.

Mutations in the \textit{Mtb} \textit{gyrB} gene were also associated with FQ resistance but at a much lower frequency. In this study, these mutations were only evaluated in a few hundred FQ resistant strains and were rare (1–2% of FQ isolates observed). Mutations in \textit{gyrB} typically occur in association with \textit{gyrA} mutations [11–15] and most often occur in codons 500 and 538[16], making it difficult to assess their individual contributions to phenotypic resistance. In a recent study by Malik et al.[21] functional genetic analysis of \textit{gyrB} indicated that certain mutations in \textit{gyrB} confer FQ resistance, however the level and pattern of resistance varied among the different mutations. Nonetheless, the results from their study provide support for the inclusion of mutations in the QRDR of \textit{gyrB} in next generation molecular assays used to detect FQ resistance in \textit{Mtb}. In this review, some \textit{gyrB} mutations did occur independently of \textit{gyrA} mutations which could help explain the phenotypic resistance in isolates that don’t have mutations in the QRDR region of \textit{gyrA}. In our study, the most common \textit{gyrB} mutations occurred in codons 500, 538, 539 and 543. No susceptible isolates were reported to contain \textit{gyrB} mutations, suggesting these rare mutations are highly specific markers of FQ-resistance.

Although rare, \textit{gyrA} double mutations were found to occur in codons 90 and 94. Double mutations suggest \textit{Mtb} may be undergoing adaptive evolution to improve the fitness of the bacteria in response to global FQ treatment [65]. Although the data from this review were limited

| Country | Mutation | # Resistant Isolates Examined | # Susceptible Isolates Examined | # Resistant Isolates with Mutation | # Susceptible Isolates with Mutation | Frequency of Mutation among Resistant Isolates | Frequency of Mutation among Susceptible Isolates |
|---------|----------|-----------------------------|-------------------------------|----------------------------------|-------------------------------------|----------------------------------------------|-----------------------------------------------|
| Vietnam (n = 3) | A90V | 192 | 40 | 37 | 0 | 0.19 | 0.00 |
| | D94G | 192 | 40 | 48 | 0 | 0.25 | 0.00 |
| | D94A | 192 | 40 | 20 | 0 | 0.10 | 0.00 |
| | D94N | 192 | 40 | 3 | 0 | 0.02 | 0.00 |
| | S91P | 192 | 40 | 2 | 0 | 0.01 | 0.00 |
| | D94Y | 192 | 40 | 7 | 0 | 0.04 | 0.00 |
| | D94H | 192 | 40 | 1 | 0 | 0.01 | 0.00 |

NA = Not applicable, division by zero

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Table 5. (Continued)
by the lack of geographical diversity of strains with double mutations, double gyrA mutations were never reported in FQ susceptible *Mtb* strains and are likely highly specific markers of FQ resistance in *Mtb*.

In this study, we noted that ofloxacin-resistant clinical isolates were consistently cross-resistant to the newer FQs (e.g. moxifloxacin). While there is building evidence to suggest that certain gyrA mutations are associated with differential cross resistance to the different FQs, it would appear from our study that many of the canonical gyrA mutations should probably be considered broadly cross resistant while evidence of mutation-specific differential resistance is being verified.

The WHO has listed 27 “high burden” TB countries; data from seven of these countries (China, India, Pakistan, Philippines, Russia, South Africa and Vietnam) were included in this review. While several studies have commented on potential geographic differences [2, 15, 28, 40, 42, 45, 46, 51, 66–68] in frequencies of resistance conferring gyrA and gyrB mutations within and between countries, few attempts have been made to characterize these differences. In our study, we demonstrated that single gyrA mutations and resistance to FQs varies geographically. One possible reason for the diversity of mutations between countries may be attributed to different social and geographic transmission environments giving rise to different pressures of natural selection. A second possible reason for this diversity may be attributed to differences in treatment regimens containing FQs, which can result in geographically diverse drug-based selection pressures. Identifying geographical areas with high frequencies of unique mutations may help improve molecular surveillance methods and identify areas of concern for molecular diagnostic assay scale up. However, as long as next generation molecular diagnostics or whole gene/genome approaches are able to detect all of the canonical gyrA mutations known to confer resistance, and geographically diverse mutations show the same specificity, the observed spatial diversity of mutations will not decrease sensitivity or specificity of next generation assays.

The WHO Stop TB Program has emphasized the need to strengthen diagnostic testing and the need to develop rapid diagnostics [69]. The only commercial assay for rapid detection of FQ resistance in clinical samples currently is the MTBDRsl line probe assay (Hain Lifescience, Nehren, Germany). The MTBDRsl assay can detect *Mtb* mutations A90V, S91P, D94A, D94N/Y, D94G, and D94H, with a recently reported pooled sensitivity and specificity of 87% and 97% respectively on direct clinical samples [70]. While we did observe mutations in gyrA outside of the codons interrogated by the MTBDRsl assay, and in gyrB (1–2% of FQ-resistant strains showed single mutations in gyrB), our findings indicate that at least 85% and 82% of moxifloxacin and ofloxacin resistant strains, respectively, were observed to contain mutations in the codons interrogated by the MTBDRsl assay. This data is consistent with the pooled sensitivity of the MTBDRsl assay recently reported in a Cochrane review [71] and suggests that the MTBDRsl assay is likely to have good sensitivity for detection of moxifloxacin and ofloxacin resistance globally depending on its ability to detect these mutations in clinical samples. Based on the frequency of QRDR mutations observed in FQ resistant strains in China and Russia (83% and 84% respectively), the MTBDRsl assay may have a similar sensitivity in those countries. However, it is important to understand that biases in the collection of strains in the studies from those countries may have contributed to the frequencies observed. This emphasizes the need for representative national and global surveillance of resistance mutations to obtain more reliable estimates of global frequencies of these mutations in order to design next generation molecular diagnostics and optimize global performance.

Recently the WHO Expert Group concluded that based on available evidence, the GenoType MTBDRsl assay had a pooled sensitivity and specificity of 84% and 97% respectively. The expert panel determined that while the specificity was sufficient for a “rule-in” test of FQ resistance, it should not be used as a replacement test for conventional phenotypic testing yet [72]
due to a high proportion of phenotypic FQ resistant isolates that it appears to be unable to detect. Our review of the global frequencies of gyrA mutations in FQ resistant isolates suggests that next generation assays able to detect all of the gyrA mutations presented in this review should have sensitivities of at least 87% and 83% for detection of moxifloxacin and ofloxacin resistance respectively, depending on their ability to detect these mutations in clinical samples. Based on our review and previously published work on gyrA frequencies by others [16, 73, 74], it seems unlikely that molecular diagnostics based on gyrA mutations alone will have global sensitivities exceeding 95%, and may suffer from geographic variability. But it is important to view this limitation in the context of the fact that less than 30–45% of MDR-TB, and likely less FQ resistant TB, is currently being detected by standard phenotypic methods [75]. Existing molecular diagnostics based on detection of QRDR mutations could significantly improve the number of FQ resistant TB cases being detected and treated appropriately.

Limitations
This study has several limitations. The cumulative frequencies calculated were based on two main assumptions. First, it was assumed that all the mutations reported were independent of each other. If some isolates were misclassified as independent when they were, in fact, not, this could have caused an overestimation in our cumulative frequencies of that specific mutation. Every effort was made to ensure that the isolates and the mutations presented in one study were not also reported in another study. Every manuscript was scrutinized for evidence of the same isolates being reported on and to the best of our knowledge all isolates reported were unique. A second potential source of misclassification error was in our use of the DST results as reported. For example, if an isolate was misclassified as resistant based on faulty DST data, when it was, in fact, susceptible, and it did not have the expected mutation then we would have underestimated the cumulative frequency of that mutation among resistant isolates. To minimize the chances of such misclassification, we excluded manuscripts with no explicit descriptions of their DST methods and clear definitions of what constituted a resistant or susceptible isolate using accepted DST drug concentrations and methodologies. For those studies that did not state which section of a gene was sequenced, this was assumed based on the mutations reported, possibly introducing misclassification bias. Identified “hot spots” were grouped by country (as not all studies reported the city the isolates were collected in) regardless of the year the isolates were collected. Additionally it was assumed that these mutations would remain in the same locations between the time the data were collected and the time of this publication. Moreover, studies reporting from only one country were generalized to the entire country, possibly introducing misclassification bias. Lastly, the exclusion of laboratory generated mutations may have led to the under-reporting of gyrA mutations. While laboratory generated mutations and clinical isolates have common features, they also have some key differences. Sun et al. [27] observed mutations occurring in clinical isolates most often did not occur in the laboratory generated mutations. Furthermore, clinical isolates and laboratory generated mutations differed in frequency for various mutation patterns. Thus, while laboratory generated mutations are critical to the understanding of the mechanism of mutations, these mutations do not always accurately reflect the mutations and frequencies of mutations observed in clinical isolates and were therefore excluded from this review of mutations for the purposes of understanding molecular diagnostics for clinical isolates.

Conclusion
To maximize the sensitivity and specificity of molecular diagnostics based on detection of mutations conferring FQ resistance in Mtb, we need an understanding of the frequency and
geographic distribution of these mutations. In this review, gyrA mutations reported in codons 88–94 appeared to account for at least 82% of phenotypic ofloxacin resistance and 85% of moxifloxacin resistance globally, while gyrB mutations and gyrA double mutations occurred only rarely. While we did observe geographic differences in the frequencies of specific gyrA mutations between countries, it is likely that next generation molecular assays that can detect all of the gyrA and gyrB mutations documented to confer resistance, will have good sensitivity and specificity globally. Using existing molecular diagnostics to rapidly detect FQ resistance in clinical Mtb strains could substantially enhance drug resistance control efforts, with the goal of interruption of disease transmission and ultimately incidence reduction, especially in countries with cross-resistance. While it appears the line probe assay, Genotype MTBDRsl should have good sensitivity and specificity for detecting phenotypic FQ resistance globally, future national and international surveillance studies focusing on prevalence of mutations across all of gyrA and gyrB, could improve design and optimization of next generation molecular diagnostics for detecting FQ resistance.

Supporting Information

S1 Table. List of all mutations not meeting criterion for inclusion. (DOC)

S2 Table. PRISMA Checklist. (DOC)

S3 Table. Cumulative Frequencies of the Most Frequently Occurring Double Mutations within gyrA Gene among Mycobacterium tuberculosis Isolates Resistant to Fluoroquinolones. (DOCX)

Author Contributions

Conceived and designed the experiments: EA DC AC TG TR SB JA. Performed the experiments: EA DC TR. Analyzed the data: EA DC. Contributed reagents/materials/analysis tools: EA DC. Wrote the paper: EA DC AC TG TR SB JA.

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