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Increased prevalence of levofloxacin-resistant *Mycobacterium tuberculosis* in China is associated with specific mutations within the *gyrA* gene

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**Highlights**
· An alarming increase in prevalence of FQ-resistant TB is observed in China over the past decade.

· This dynamic change in FQ resistance is majorly attributed to the increase of high-level LFX-resistance.

· A significant difference is noted in the proportion of LFX-resistant isolates harboring specific mutations within *gyrA* gene between 2005 and 2015.

**Abstract**

*Objectives:* To compare the prevalence of levofloxacin (LFX) resistance and the population structure of *Mycobacterium tuberculosis* (MTB) with different mutations conferring LFX resistance between 2005 and 2015.

*Methods:* 542 MTB isolates were randomly selected from pulmonary TB patients in 2005 and 2015 were analysed for minimum inhibitory concentrations (MICs) and quinolone resistance-determining regions (QRDR) respectively.

*Results:* A total of 542 MTB isolates were analyzed, of which 111 (20.5%) were resistant to LFX. Of 42 and 69 LFX-resistant isolates from 2005 and 2015 respectively, 40.6% (28/69) had MIC high-level LFX resistance in 2015, which was significantly higher than 16.7% (7/42) in 2005 (*P* = 0.02). There are 87 (78.4%) mutations of these 111 LFX-resistant isolates. In addition, a significant difference in proportion was observed in the isolates with mutations in codon 90 of the *gyrA* gene between 2005 and
2015 (11.9% in 2005 versus 29.0% in 2015, \( P = 0.04 \)).

**Conclusion:** The prevalence of LFX-resistant TB in China was an alarming increase between 2005 and 2015. This dynamic change is majorly attributed to the increase of high-level LFX-resistance. Moreover, a significant difference was noted in the proportion of LFX-resistant isolates harboring specific mutations within the gyrA gene between 2005 and 2015.

**Keywords:** tuberculosis; levofloxacin; drug resistance; gyrA; China

**Introduction**

The global control of tuberculosis (TB) remains a major challenge due to the epidemic of drug-resistant tuberculosis, especially multidrug-resistant tuberculosis (MDR-TB), which is resistant to rifampin and isoniazid (Zhao et al., 2012; Zhang et al., 2014). According to recent WHO global estimates, there were 480,000 MDR-TB cases in 2017 (WHO, 2018). Almost half of the global MDR-TB cases occurred in China and India (WHO, 2018). Due to resistance to both of the most effective antituberculosis drugs, the treatment of MDR-TB requires the use of the less effective and more toxic second-line drugs; approximately half of MDR-TB patients achieve favorable outcomes by the end of prolonged treatment (Velasquez et al., 2014; Xu et al., 2018). The high proportion of treatment failure facilitates the transmission of this severe form of TB in the community (Velasquez et al., 2014). Early detection of MDR-TB and initiation of proper regimens with second-line drugs are essential to reduce the high mortality associated with this
disease (van Cutsem et al., 2016).

Fluoroquinolones (FQs) are considered to be the cornerstone of treatment regimens for MDR-TB (Ginsburg et al., 2003; Falzon et al., 2013). The antimicrobial action of FQs is executed through the inhibition of DNA synthesis by targeting DNA gyrase in *Mycobacterium tuberculosis* (MTB) (Ginsburg et al., 2003; Zhang et al., 2014a). The DNA gyrase of MTB consists two A and two B subunits encoded by *gyrA* and *gyrB*, respectively (Zhang et al., 2014a). Mutations in *gyrA* or *gyrB* lead to the acquired resistance to FQ, which have been widely used as predictive markers for FQ resistance in molecular diagnostics (Tagliani et al., 2015; Pang et al., 2016). A wide variety of mutations within these two genes have been identified, and different substitutions in the gyrase genes are associated with different FQ resistance levels in MTB isolates (Li et al., 2014; Willby et al., 2015). In addition to classical gene mutations, other mechanisms are presumed to be involved in FQ resistance, such as blockage by the permeability barrier and efflux of the drug from the cell (Lata et al., 2015a; Lata et al., 2015b). Notably, the frequency of *gyrA* and *gyrB* genes conferring FQ-resistance exhibits great diversity across studies from various regions (Zhang et al., 2014a), which majorly impacts the diagnostic accuracy of molecular methods for FQ resistance.

China is one of the “hotspot” regions for drug-resistant TB (Zhao et al., 2012). A recent hospital-based study concerning drug-resistant TB revealed that about one third of TB cases seeking health care in Beijing were infected with MDR-TB (Pang et al.,
Although FQs are the most effective drugs used in the chemotherapy of MDR-TB, they have been widely used in the treatment of respiratory, gastrointestinal, and urinary tract bacterial infections over the past few decades in China (Zhang et al., 2014a). The poorly controlled use of FQs contributes to the emergence of acquired resistance for TB patients, thereby resulting in transmission of strains that are already FQ-resistant. Molecular epidemiological studies have demonstrated that resistance to moxifloxacin increased significantly in China between 2000 and 2010 (Pang et al., 2017c). Similar results were observed by our recent report which showed a significant increase in the prevalence of levofloxacin (LFX) resistance by comparative analysis of MTB isolates collected between 2005 and 2015 (Huo et al., 2018). To clarify the prevalence of LFX resistance and population structure of MTB with different mutations conferring LFX resistance, we analyzed the LFX minimal inhibitory concentration (MIC) distribution and the mutation types of MTB isolates randomly collected from the National Clinical Center on Tuberculosis in China.

**Materials and methods**

**Ethics Statement**

The protocols of this study were approved by the Ethics Committee of the Beijing Chest Hospital, Capital Medical University. As the patient records were anonymized and de-identified before analysis, informed consent was not obtained from participants.
Strains and culture conditions

In this study, 542 MTB isolates were randomly selected from pulmonary TB patients diagnosed in Beijing Chest Hospital in 2005 and 2015 using a simple random sampling method, including 273 and 269 isolates from 2005 and 2015, respectively. All of the isolates were collected from unduplicated patients. Of these isolates, 111 were resistant to LFX (42 from 2005 and 69 from 2015), determined by the absolute concentration method as previously described (Canetti et al., 1969). Conventional drug susceptibility testing results were obtained from routine laboratory data. The concentration was 2 mg/L in Löwenstein–Jensen (LJ) medium. All LFX-resistant isolates were subcultured on LJ medium for four weeks at 37 °C for further analysis.

Minimum inhibitory concentration

The microplate Alamar blue assay (MABA) was used to determine the MICs of LFX-resistant MTB isolates identified by conventional drug susceptibility testing as previously reported (Franzblau et al., 1998). Briefly, freshly grown colonies were harvested from the surface of LJ slants and transferred to sterile screw-cap tubes containing glass beads and one mL normal saline. After vigorous agitation on a vortex mixer for one minute, the suspensions were adjusted to a 1.0 McFarland turbidity standard. Lastly, 100 μL of the 1:20 diluted inoculum was added to all wells, and the plates were sealed and incubated for seven days. For the MIC assays, 70 μL of AlamarBlue solution was added and reexamined after 24 hours for color development. Visual observation of a color change from blue to pink indicated bacterial growth. The
MIC was defined as the lowest concentration of a drug that would inhibit bacterial growth. The LFX concentrations tested ranged from 2.0 to 32.0 mg/L. The reference *M. tuberculosis* H37Rv (ATCC 27249) was tested in each batch experiment for quality control purposes, and the LFX concentrations tested were from 0.063 to 1.0 mg/L. All experiments were done in duplicate to ensure the accuracy of the MIC results. If the isolates gave discordant MIC results, we conducted the following repeated experiments till obtainment of three identical MIC values, which were used as the MIC values of the corresponding isolates. According to the definition of high-level moxifloxacin, the value (2.0 mg/L) four times greater than the susceptibility breakpoint of 0.5 mg/L was set as breakpoint concentration for high-level resistance. Hence, the MIC breakpoint concentration was defined as eight mg/L for high-level LFX resistance.

**DNA amplification and sequencing**

Extraction of crude genomic DNA from MTB isolates was performed using the boiling method (Zhang et al., 2015). One loop of freshly cultured mycobacteria was transferred into a microcentrifuge tube with 500 μL Tris-EDTA buffer. Following centrifugation at 13,000 rpm for two minutes, the pellet was resuspended in 500 μL Tris-EDTA buffer and then heated in a 95 °C water bath for one hour. The supernatant was used as template for PCR amplification.

Partial fragments of the *gyrA* and *gyrB* genes containing quinolone resistance-determining regions (QRDR) were amplified among LFX-resistant MTB isolates. The
primer pairs for \textit{gyrA} and \textit{gyrB} were synthesized according to a previous report (Pang et al., 2017b). Briefly, a solution of 50 μL PCR reaction mixture was prepared containing 25 μl 2X PCR Master Mixture, 2 μL of DNA template, and 0.2 μM of each primer set. Amplification was conducted with an initial denaturation at 95 °C for five minutes, followed by 35 cycles of amplification (denaturation at 95 °C for one minute, annealing at 60 °C for one minute, and extension at 72 °C for one minute, with a final extension at 72 °C for five minutes). PCR products were sent to Ruibio BioTech Company (Beijing, China) for sequencing. DNA sequences were aligned with homologous sequences of the reference MTB H37Rv strain on the NCBI website (http://www.ncbi.nlm.nih.gov/BLAST).

RD207 deletion-targeted multiplex PCR was used to identify Beijing genotype strains. To distinguish between modern and ancient Beijing genotype strains, we detected whether the IS6110 fragment was present within the noise transfer function region as described previously (Huo et al., 2018).

\textbf{Statistical analysis}

Categorical variables are presented as numbers and percentages. The distribution of categorical variables was compared using Pearson's chi-square test or Fisher's exact test. The difference was considered statistically significant if the two-tailed $P$ value was less than 0.05. All statistical analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, Illinois, USA).
Results

Demographics and risk factor characteristics of LFX-resistance

Table 1 summarizes patient characteristics of LFX-resistant cases compared with those of LFX-susceptible cases. The distribution of LFX-resistant cases differed among different age groups. When elderly patients (>64 years old) were set as the control group, patients aged <25 years old and 25-44 years old were more likely to have LFX-resistant TB (patients <25 years old had an odds ratio of 3.23 with a 95% confidence interval of 1.26-8.28, while patients 25-44 years old had an odds ratio of 4.38 with a 95% confidence interval of 2.00-9.62). Patients with previous treatment history had significantly higher odds of being affected by LFX-resistant TB compared with new cases (odds ratio: 4.09, 95% confidence interval: 2.64-6.32). In contrast, sex and Beijing genotype had no significant influence on the distribution of LFX-resistant TB among the groups ($P > 0.05$).

MICs of LFX-resistant isolates

A total of 111 LFX-resistant isolates were analyzed in order to determine MICs, including 42 isolates (37.8%) from 2005 and 69 isolates (62.2%) from 2015. The MICs of MTB isolates against LFX are shown in Figure 1. Of the 42 LFX-resistant isolates from 2005, seven (16.7%) had MIC values higher than four mg/L, defined as high-level LFX resistance. Of the 69 LFX-resistant isolates collected in 2015, 28 (40.6%) were
classified as having high-level LFX resistance, which was significantly higher than that in 2005 ($P = 0.02$).

**Mutations conferring LFX resistance**

The fragments of the *gyrA* and *gyrB* genes containing QRDRs were analyzed. DNA sequence chromatograms showed the presence of mutations in 87 (78.4%) of these 111 LFX-resistant isolates. Among these 87 isolates, 82 (94.3%) carried single mutations of *gyrA*, and the remaining five (5.7%) harbored double mutations in *gyrA* and *gyrB*.

As shown in Figure 2 (Table S1), the most prevalent mutation was codon 94 of *gyrA* (51.4%), resulting in the amino acid substitution of Asp with Gly (29.7%), Ala (13.5%), Tyr (4.5%), or Asn (3.6%). As the second most affected codon, mutations in codon 90 of *gyrA* accounted for 25 LFX-resistant isolates. In addition, five isolates had a mutation in codon 91 and one in codon 88 of *gyrA*.

Results of MIC$_{50}$ and MIC$_{90}$ for different mutation profiles are summarized in Figure 2 (Table S1). Isolates with the mutations Ser91Pro and Asp94Ala had an MIC$_{90}$ of 4.0 mg/L for LFX, whereas Ala90Val, Asp94Gly, and Asp94Tyr were found to have a MIC$_{90}$ of 8.0 mg/L for LFX. In addition, we observed that the Asp94Asn mutation was associated with high-level LFX-resistance. Of the five isolates harboring this mutation, three had MICs of 16.0 mg/L and two had MICs of 32 mg/L for LFX.

We further compared the distribution of mutation profiles conferring LFX resistance
between 2005 and 2015. As shown in Table 2, out of 42 LFX-resistant isolates in 2005, 29 (69.0%) carried mutations within the gyrase genes, whereas the proportion of LFX-resistant isolates harboring these mutations in 2015 was 84.1% (58/69). A significant difference in mutations between 2005 and 2015 was observed in codon 90 of the \( \text{gyrA} \) gene of the isolates (11.9% in 2005 versus 29.0% in 2015, \( P = 0.04 \)). In contrast, the percentage of isolates with other mutations showed no significant difference between the two years (\( P > 0.05 \)).

**Association between gyrase mutations and genotypes**

The distribution of mutation types among different genotypes is listed in Table 3. There were no significant differences in the gyrase mutation distribution between modern and ancient Beijing genotype isolates (\( P > 0.05 \)). When using non-Beijing genotype isolates as the control, the proportion of isolates harboring \( \text{gyrA} \) mutations among Beijing genotypes was significantly higher (82.2%; \( P = 0.01 \)), whereas the LFX-resistant isolates belonging to non-Beijing genotypes were more likely to have no mutations within the QRDR of gyrase genes than Beijing genotypes (17.8% for Beijing genotypes versus 60.0% for non-Beijing genotypes, \( P = 0.01 \)).

**Discussion**

Fluroquinolones remain the most effective antimicrobial agent used in the chemotherapy of MDR-TB (Ginsburg et al., 2003). Our study has demonstrated an alarming increase in the prevalence of LFX-resistant TB in China over the past decade.
Notably, this dynamic change is majorly attributed to the increase of high-level LFX-resistance. It can be assumed that the emergence of highly LFX-resistant bacteria is associated with the accumulation of gyrA mutations due to spatial and temporal antibiotic concentration gradients occurring in a treated organism (Palmer and Kishony, 2013). The poorly controlled use of FQs in empirical treatment of respiratory infections in China may thus play an important role in prompting progressive selection toward high-level resistance (Li, 2014). A recent report by Rigouts and colleagues revealed that the presence of gyrA mutations conferring high-level FQ resistance could predict poor treatment outcome in MDR-TB patients (Rigouts et al., 2016). Hence, the increasing prevalence of LFX-resistant TB, especially high-level resistant TB, highlights the potential for the high rate of adverse clinical outcomes for patients infected with MDR-TB, further promoting transmission of this disease in the community. More efforts are needed to address the misuse of FQs in China, which is essential to prevent the emergence and transmission of high-level FQ-resistant tubercle bacilli.

Resistance to FQs in clinical MTB isolates commonly arises through the mutation of the gyrA gene (Zhang et al., 2014a). In the present study, we observed that 78.4% of LFX-resistant isolates harbored mutations in the QRDR of gyrA, similar to those in Russia (83%), Shanghai (76%) and Guangdong (73%) (Yin and Yu, 2010; Andersson and Levin, 1999; Zhang et al., 2015). Similar to previous studies (Zhang et al., 2014a; Andersson and Levin, 1999), substitutions at codon 94 were the most prevalent among the LFX-resistant strains in China. In contrast, we observed that 21.6% of LFX-resistant
isolates harbored no mutations in the QRDR of \textit{gyrA} and \textit{gyrB}. Besides the permeability barrier and mycobacterial efflux pump, it is becoming increasingly clear that the inadequate limit-to-detection provided by Sanger sequencing to detect heteroresistance may be an important explanation for these discordant results between genotype and phenotype (Thomas, et al., 2016).

Notably, a significant difference was observed in the proportion of LFX-resistant isolates harboring mutations at codon 90 of the \textit{gyrA} gene between 2005 and 2015. Traditionally, antibiotic resistance is accompanied by a fitness cost, especially when it occurs via a mutation of genes that are essential for bacterial survival (Andersson and Levin, 1999). The occurrence of FQ resistance due to mutations in gyrase genes could also lead to a defect in the gyrase function, perhaps affecting the rate of the fidelity of DNA synthesis. Therefore, the \textit{gyrA} mutations conferring high-level resistance may be associated with a high fitness loss. Although high-level resistance favors bacteria survival under exposure to antibiotics, its high fitness cost could confer a disadvantage in the absence of drug pressure.

The Beijing MTB genotype was previously associated with an increased prevalence of the specific mutation conferring rifampin and isoniazid resistance in several regions of the world (Hillemann et al., 2005; Lipin et al., 2007; Zhang et al., 2015). Similarly, we found that the Beijing genotype of LFX-resistant isolates were significantly more likely than LFX-resistant isolates of other genotypes to harbor \textit{gyrA} mutations in a
setting where the Beijing genotype is prevalent; this corroborates a previous report from Vietnam (Duong et al., 2009). Numerous studies have demonstrated that Beijing genotype strains carry alterations in putative mutator genes, resulting in altered DNA repair and an increased mutation rate (Parwati et al., 2010; Shitikov et al., 2017). Owing to this inherent characteristic, Beijing genotype strains may be more prone to accumulating genetic mutations after exposure to anti-TB drugs, which may be a potential explanation for our findings. Moreover, considering the fact that the proportion of the Beijing genotype significantly increased over the past decade (Pang et al., 2017c), our results highlight that the increasing prevalence of this specific genotype could drive the epidemic spread of LFX-resistant TB in China.

We also acknowledge several limitations to this study. First, the strains analyzed in this study were only acquired from the National Clinical Center on Tuberculosis of China rather than from multiple centers. In addition, as a subsequent study of our previous findings, we did not conduct statistical justification for the decision of MTB isolate number. This bias in sample selection may limit the extension of our conclusions for other settings. Second, the isolates in this study were obtained in the hospital setting, which results in greatly increasing proportion of previously treated cases. Further studies analyzing the MTB isolates from community settings are required to elucidate our hypothesis. Third, although we speculate that the increasing prevalence of LFX-resistant TB may lead to a high rate of adverse clinical outcomes and its subsequent transmission in China, the clinical outcomes of TB patients were not collected in this
study due to low follow-up rates, given that the patients came from various regions of China. Despite these limitations, our findings indicate that more attention should be given to the increasing prevalence of LFX-resistant TB in China, driven by specific mutations within the *gyrA* gene.

In conclusion, our data demonstrate that an alarming increase in the prevalence of LFX-resistant TB in China has occurred between 2005 and 2015. This dynamic change is majorly attributed to the increase of high-level LFX-resistance. In addition, a significant difference is noted in the proportion of LFX-resistant isolates harboring mutations at codon 90 of the *gyrA* gene between 2005 and 2015. Further studies are urgently needed to elucidate the underlying mechanism of the increase in prevalence of LFX-resistant TB driven by the specific mutations within the *gyrA* gene in China.

**Acknowledgements**

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**Author contributions**

FH, FZ and YP designed this study. FH, FZ, YX, YS, QL, YL and YM performed experiments. FH, FZ and LJ interpreted the data. FH, FZ and YP wrote the manuscript.
All authors approved the final version of the paper.

Conflict of interest

None.
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Li Y. China's misuse of antibiotics should be curbed. BMJ 2014; 348: g1083.


Clinical Microbiology 2015; 53: 2961-2969.


Figure Legends

Figure 1 Comparison of MTB isolates with different LFX MICs between 2005 and 2015

(A) Distribution of MTB isolates with different LFX MICs between 2005 and 2015; (B) Comparison of MTB isolates with high- and low-level resistance between 2005 and 2015. High-level resistance represents the isolate with an MIC value \( \geq 8 \) mg/L; Low-level resistance represents the isolate with an MIC value \( \leq 4 \) mg/L.

Figure 2 Association between mutations in gyrA and gyrB gene and LFX MICs
Table 1 Distribution of LFX resistant MTB isolates stratified to demographic and clinical characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. (%) of isolates</th>
<th>OR&lt;sup&gt;a&lt;/sup&gt; (95%CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LFX-resistant (&lt;i&gt;n&lt;/i&gt;=111)</td>
<td>LFX-susceptible (&lt;i&gt;n&lt;/i&gt;=431)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>74(66.7)</td>
<td>266(61.7)</td>
<td>1.24(0.80-1.93)</td>
</tr>
<tr>
<td>Women</td>
<td>37(33.3)</td>
<td>165(38.3)</td>
<td>1.00(Ref.)</td>
</tr>
<tr>
<td>Age group (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>14(12.6)</td>
<td>45(10.4)</td>
<td>3.23(1.26-8.28)</td>
</tr>
<tr>
<td>25-44</td>
<td>60(54.1)</td>
<td>142(32.9)</td>
<td>4.38(2.00-9.62)</td>
</tr>
<tr>
<td>45-64</td>
<td>29(26.1)</td>
<td>161(37.4)</td>
<td>1.87(0.82-4.27)</td>
</tr>
<tr>
<td>&gt;64</td>
<td>8(7.2)</td>
<td>83(19.3)</td>
<td>1.00(Ref.)</td>
</tr>
<tr>
<td>Treatment History</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New case</td>
<td>44(39.6)</td>
<td>314(72.9)</td>
<td>1.00(Ref.)</td>
</tr>
<tr>
<td>Re-treated</td>
<td>67(60.4)</td>
<td>117(27.1)</td>
<td>4.09(2.64-6.32)</td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beijing</td>
<td>101(91.0)</td>
<td>391(90.7)</td>
<td>1.03(0.50-2.14)</td>
</tr>
<tr>
<td>Non-Beijing</td>
<td>10(9.0)</td>
<td>40(9.3)</td>
<td>1.00(Ref.)</td>
</tr>
</tbody>
</table>

<sup>a</sup>OR: odds ratio; CI, confidence interval.
Table 4: Comparison of different mutations in gyrA and gyrB gene between 2005 and 2015

<table>
<thead>
<tr>
<th>Year (n)</th>
<th>gyrA88</th>
<th>gyrA90</th>
<th>gyrA91</th>
<th>gyrA94</th>
<th>gyrB499</th>
<th>gyrB512</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005 (42)</td>
<td>0(0.0)</td>
<td>5(11.9)</td>
<td>3(7.1)</td>
<td>21(50.0)</td>
<td>1(2.4)</td>
<td>0(0.0)</td>
<td>29(69.0)</td>
</tr>
<tr>
<td>2015 (69)</td>
<td>1(1.4)</td>
<td>20(29.0)</td>
<td>2(2.9)</td>
<td>36(52.2)</td>
<td>1(1.4)</td>
<td>2(2.9)</td>
<td>58(84.1)</td>
</tr>
</tbody>
</table>

P value | 1.00 | 0.04 | 0.57 | 0.82 | 1.00 | 0.52 | 0.06 |
### Table 5 Distribution of MTB isolates with different mutations between Beijing and non-Beijing genotype sublinages

<table>
<thead>
<tr>
<th>Mutation type</th>
<th>No. of isolates with different mutations (%)</th>
<th>Beijing (n=101)</th>
<th>Non-Beijing (n=10)</th>
<th>Total (n=111)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ancient (n=23)</td>
<td>Modern (n=78)</td>
<td>P value</td>
<td>Total (n=101)</td>
</tr>
<tr>
<td>GyrA</td>
<td>17(73.9)</td>
<td>66(84.6)</td>
<td>0.39</td>
<td>83(82.2)</td>
</tr>
<tr>
<td>Gly88Ala</td>
<td>0(0)</td>
<td>1(1.3)</td>
<td>1.00</td>
<td>1(1.0)</td>
</tr>
<tr>
<td>Ala90Val</td>
<td>3(13.0)</td>
<td>21(26.9)</td>
<td>0.42</td>
<td>24(23.8)</td>
</tr>
<tr>
<td>Ser91Pro</td>
<td>2(8.7)</td>
<td>3(3.8)</td>
<td>0.58</td>
<td>5(5.0)</td>
</tr>
<tr>
<td>Asp94Asn</td>
<td>1(4.3)</td>
<td>3(3.8)</td>
<td>1.00</td>
<td>4(4.0)</td>
</tr>
<tr>
<td>Asp94Ala</td>
<td>3(13.0)</td>
<td>11(14.1)</td>
<td>1.00</td>
<td>14(13.9)</td>
</tr>
<tr>
<td>Asp94Gly</td>
<td>6 (26.1)</td>
<td>25(32.1)</td>
<td>0.88</td>
<td>31(30.7)</td>
</tr>
<tr>
<td>Asp94Tyr</td>
<td>2(8.7)</td>
<td>3(3.8)</td>
<td>0.56</td>
<td>5(5.0)</td>
</tr>
<tr>
<td>GyrB</td>
<td>1(4.3)</td>
<td>3(3.8)</td>
<td>1.00</td>
<td>4(4.0)</td>
</tr>
<tr>
<td>Asn499Thr</td>
<td>0(0)</td>
<td>2(2.6)</td>
<td>1.00</td>
<td>2(2.0)</td>
</tr>
<tr>
<td>Gly512Arg</td>
<td>1(4.3)</td>
<td>1(1.3)</td>
<td>0.41</td>
<td>2(2.0)</td>
</tr>
<tr>
<td>No mutation</td>
<td>6(26.1)</td>
<td>12(15.4)</td>
<td>0.39</td>
<td>18(17.8)</td>
</tr>
</tbody>
</table>