Incidence of Moxifloxacin Resistance in Clinical *Mycobacterium tuberculosis* Isolates in Houston, Texas

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**Keywords:** tuberculosis, moxifloxacin, quinolones, drug resistance

**Running title:** moxifloxacin resistant tuberculosis

**Text word count:** 2,051

**Abstract word count:** 188

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**Funding:** The project has been funded with federal funds through the National Institutes of Health, Grant 5R03AI74647-2, Principal Investigator: Hana El Sahly

**Conflict of Interest:** The authors do not have commercial or other associations that might pose a conflict of interest with the research presented in this manuscript.
Abstract

Comprehensive data on the prevalence of quinolone resistance in *Mycobacterium tuberculosis* (MTB) clinical isolates in the US are scarce. Using a systematic population-based approach, MTB strains from tuberculosis (TB) cases were collected in Harris County, Texas, 2007-2008. MTB isolates’ susceptibility to moxifloxacin and ofloxacin was determined with the agar proportion indirect susceptibility method. Spoligotype and MIRU12-based genotyping of MTB isolates and sequencing of the *gyrA, gyr B, Rv2686c, Rv2687c* and *Rv2688c* gene in quinolone-resistant and year-of-diagnosis-matched MTB isolates were performed. Susceptibility testing on 557 MTB isolates was performed, of which 10 (1.8%) were resistant to moxifloxacin. There was a 100% concordance between ofloxacin and moxifloxacin susceptibility. A quinolone was prescribed to at least 5 (50%) patients in the period preceding TB diagnosis. Multidrug resistant tuberculosis was significantly associated with quinolone resistance (*P*<0.01). Mutations in the quinolone resistance determining region of *gyrA* were found in 50% of the resistant isolates. No other presumptive quinolone-resistance-associated mutations were identified. We conclude that the incidence of moxifloxacin-resistant TB is low in Harris County and is associated with MDR-TB. Previous exposure to quinolones is common among patients with moxifloxacin resistance and warrants more careful evaluation.
The anti-tuberculous drug pipeline is extremely slow: it has been more than 40 years since rifampin was introduced for wide clinical use. While fluoroquinolones were initially indicated and widely used for non-mycobacterial infections, their anti-tuberculous properties were recognized early and described in the literature (16). Their tolerability and relative low to moderate cost have made them an attractive target for development as anti-tuberculous drugs. The newer generation of quinolones, such as sparfloxacin, gatifloxacin and moxifloxacin have better in vitro activity against *Mycobacterium tuberculosis* (MTB) clinical isolates than older generation quinolones, such as ofloxacin, levofloxacin and ciprofloxacin, as suggested by lower minimum inhibitory concentration (MIC), higher peak serum concentration to MIC ratio and 24-hour area under the curve to MIC ratio (11, 22-29, 36). In vivo studies in animal models and humans have corroborated these findings (19, 34). Due in part to the side effects observed with sparfloxacin (phototoxicity) and gatifloxacin (dysglycemia), moxifloxacin has been the quinolone to reach the furthest in clinical antimycobacterial testing (4). Plans for further developing moxifloxacin as an anti-tuberculous agent to shorten the course of TB chemotherapy are underway and clinicians are using the medication when there is intolerance or resistance to first-line antituberculous agents. However, there exists another dynamic that can potentially affect the use of moxifloxacin in TB treatment: quinolones are one of the most widely prescribed antibiotics for infections other than TB. In certain TB-endemic countries, quinolones can even be purchased over the counter (20). This raises the concern that MTB resistance to moxifloxacin may develop due to reasons that are unrelated to its use as a TB treatment, thus jeopardizing the usefulness of moxifloxacin as a first-line TB drug in the future. This
is especially concerning, given the high degree of cross resistance between various quinolones in MTB strains (17, 30, 31, 35); although a debate on whether newer generation quinolones retain activity in quinolone-resistant strains is ongoing (23). Two risk factors have been associated with the development of quinolone resistance in MTB clinical isolates: prolonged or repeated exposure to quinolones prior to the diagnosis of TB and resistance to first line antituberculous drugs, especially multidrug-resistance (MDR) TB, presumably due to previous quinolone exposure (5, 6, 16, 17, 24, 35).

The MTB isolates used to evaluate quinolone resistance in many of the aforementioned studies were either from referral centers, Medicaid patients or in patients covered by certain drug benefit plans. Also, many of these studies used older generation quinolones in the susceptibility testing assays. We evaluated in a prospective, population-based methodology the incidence of moxifloxacin and ofloxacin resistance in MTB isolates collected from Harris County, Texas (referred to as Houston in the rest of the text) over a 24-month period. We also compared the isolates’ genotypes and the sequencing data of genes potentially associated with quinolone resistance between quinolone-susceptible and quinolone-resistant MTB strains, using a nested case-control approach.

Materials and Methods

MTB strains. All available MTB isolates recovered from patients diagnosed with TB in Houston between January 1st, 2007 and December 31st, 2008 were collected and sent to the Mycobacteriology Laboratory at the Texas Department of State Health Services in Austin, TX (Texas DSHS) for quinolone susceptibility testing.
Susceptibility testing. We tested the susceptibility of MTB isolates to ofloxacin and moxifloxacin using the agar proportion indirect susceptibility assay (3). MTB strains that showed ≥ 1% colony-growth at a moxifloxacin concentration of 0.5µg/ml or an ofloxacin concentration of 2.0µg/ml were considered resistant to moxifloxacin or ofloxacin, respectively. Of note, these breakpoints are used based on data in the literature that may be inconclusive. We further determined the MIC of moxifloxacin in all moxifloxacin-resistant strains. The MIC was considered to be the lowest concentration that inhibited ≥ 99% of the mycobacterial growth. Information on susceptibility to the first-line agents was collected from Tuberculosis Information Management Systems (TIMS) surveillance data managed by the Texas DSHS.

Patient information. Basic demographic and clinical data were collected from TIMS surveillance data. Detailed clinical information regarding TB cases with moxifloxacin-resistant isolates was obtained from the City of Houston Department of Health and Human Services and Harris County Public Health and Environmental Services.

MTB molecular characterization. All isolates were genotyped as part of the Centers for Disease Control and Prevention National Genotyping Project (available at http://web-tb.forum.cdc.gov). Two genotyping methodologies were used: spoligotyping and 12 loci mycobacterial interspersed repetitive units (MIRU) (18, 32). Isolates with matching MIRU and spoligotypes were defined as belonging to the same “PCR type” of isolates.

Gene sequencing. Mutations associated with moxifloxacin and ofloxacin resistance were analyzed by sequencing polymerase chain reaction products of genomic DNA of the following genes: gyrA, gyrB, Rv2686c, Rv2687c and Rv2688c. The gyrA gene was sequenced using the following forward and reverse primer pairs: cctggatgtctaacgcaacc
and aggtacgaccgcgggaat, gccgacgaagaggagacc and cgtgcctgtccacgattt,  
cgacatcgacgagatccag and gccgagaacctgatggact, gctggtgaaaaagtccaagc and  	ttcctcctcagatcgctacg. The \( \text{gyrB} \) gene was sequenced using the following forward and  
reverse primer pairs: aaacgaggccagaagatcg and cttaactttgtgcggtcag,  
cgaaaccacggaatacgact and gccgagtcaccttctacgac, ctaaggcagagagttgtt and  
gcaacgtctgtctgtcactc. The \( \text{Rv2686c} \), \( \text{Rv2687c} \) and \( \text{Rv2688c} \) genes were sequenced using  
the following forward and reverse primer pairs: ctacctgtggctgcggtact and  
gttgtgaccagcacatctcg, caggccctgaatctgttgt and cttaccttgctgtactgtcgt, gtaggtgcctcgaatgtcgt  
and tggctgccaaactaactgtg, ggcaacgaggaactgaagc and accacgtcgacaccatcat,  
aacttctgcgcagctgtag and aaagctcaccgggtatgaga, atctgcatgcccttggagta and  
agaactggtgcgaaccagga. The first two genes have been widely described in the literature as  
associated with quinolone resistance in \( \text{MTB} \). The \( \text{Rv2686c} \), \( \text{Rv2687c} \) and \( \text{Rv2688c} \)  
genes putatively express an \( \text{ABC} \) quinolone efflux pump (25). Sequencing was performed  
on all strains that were moxifloxacin resistant and on at least 2 quinolone-susceptible  
isolates for each resistant isolate, matched on the year of diagnosis.  

**Human subject protection.** The study was approved by the Institutional review board of  
Texas DSHS and Baylor College of Medicine.  

**Statistical analysis.** Patients with moxifloxacin-resistant TB were compared to patients  
with moxifloxacin-susceptible TB, with respect to sociodemographic, clinical and strain  
genotype variables using bivariate chi-square and univariate analyses. \( P \) values of \( \leq 0.05 \)  
were considered statistically significant.

**Results**
In the 24-month period of the study, there were 634 culture-positive TB cases reported in Houston. We performed quinolone susceptibility testing on 557 (87.8%) MTB isolates. There were 77 MTB isolates that did not undergo testing due to non-viability (17 isolates), contamination (22 isolates) or lack of availability of isolates (38 isolates). Resistance to moxifloxacin was found in 10 isolates (1.8%) during the study period. Five isolates had a moxifloxacin MIC of 1 µg/ml and 5 isolates had an MIC of 4 µg/ml. We found 100% concordance between resistance to moxifloxacin and ofloxacin. Although information regarding prior quinolone treatments was not always complete, we found that a quinolone was prescribed to 5 out of the 10 patients with moxifloxacin-resistant isolates within two months prior to TB diagnosis (3 patients received moxifloxacin, 1 ciprofloxacin and 1 levofloxacin). There was one documented instance of transmission of a moxifloxacin-resistant (and MDR) MTB isolate as confirmed by strain genotyping from a mother to her 3-month old child. A comparison of the demographic, clinical and strain characteristics of patients with moxifloxacin-sensitive TB and moxifloxacin-resistant TB is shown in Table 1. We found that patients with moxifloxacin resistant TB were more likely to have MDR-TB (P<0.01) but less likely to have a positive skin test (P<0.01).

**Strain genotypes.** A total of 314 PCR types were identified for the 2007-2008 MTB isolates including 78 that were shared among strains. The 10 moxifloxacin-resistant isolates belonged to 9 different PCR types. Four of these PCR types were unique to the moxifloxacin-resistant isolates and six (60%) were shared with other clustered strains (range 2-65 strains). The likelihood of belonging to a PCR type cluster was comparable between the moxifloxacin-resistant and moxifloxacin-susceptible isolates (60.0% and 60.3%, P=0.99). Four (40%) of the moxifloxacin-resistant isolates and 144 (26.4%) of
the moxifloxacin-susceptible isolates were Beijing family strains ($P=0.34$). We found no
association between higher level resistance to moxifloxacin and the Beijing genotype: 2
of the Beijing family strains had a moxifloxacin MIC of $4\mu g/ml$ and 2 had an MIC of
$1\mu g/ml$.

**Sequencing data.** We identified 4 different mutations in the quinolone-resistance-
determining region (QRDR) of $GyrA$ in 5 (50%) moxifloxacin-resistant MTB isolates
that were not found in the moxifloxacin-susceptible isolates. The 2 isolates with the
A90V mutation had a moxifloxacin MIC of $1\mu g/ml$. The isolates with the D94H, D94G
and D94A had moxifloxacin MIC of $4\mu g/ml$, $4\mu g/ml$ and $1\mu g/ml$, respectively. We did
not identify a resistance associated mutation in 5 of the moxifloxacin-resistant isolates
(50%). No other polymorphism in other regions of the $GyrA$, the $GyrB$ or the Rv2686c-
Rv2687c-Rv2688c genes existed at a higher frequency in the quinolone-resistant isolates
compared to the susceptible isolates (Table 2).

**Discussion**

Using a population-based approach, we determined the moxifloxacin susceptibility in
87.8% of the MTB strains in Houston isolated over a 24-month period. We found that the
incidence of moxifloxacin resistance is low (1.8%) in an area of low TB incidence and
that there is a statistically significant association between moxifloxacin resistance and
MDR-TB. Despite incomplete data, we found that a quinolone antibiotic was prescribed
to at least half the patients with moxifloxacin-resistant TB in the period leading up to
their TB diagnosis.
The low incidence of moxifloxacin resistance in MTB isolates in Houston is reassuring, during a time when moxifloxacin is being investigated as a first line agent. The existing level of moxifloxacin resistance is comparable to the low prevalence of MDR-TB (0.64%) in Houston (10). We found a statistically significant association between moxifloxacin resistance and MDR-TB, confirming findings from other geographic regions (5, 14, 15, 33). Hence, one can hypothesize that the usefulness of the drug will be compromised in regions of significant MDR-TB prevalence, such as certain areas of the former Soviet Union where MDR-TB constitutes up to 28% of all new cases, according to the World Health Organization statistics (available at http://www.who.int/tb/publications/global_report/2010/en/index.html).

Previous studies have found an association between multiple or prolonged exposures to quinolone and quinolone-resistant TB (6, 21). In our study, exposure to a quinolone was common in patients with quinolone-resistant TB in the period preceding their TB diagnosis. The empiric treatment of pneumonia patients with quinolones is a common and recommended practice, and may mask some of the TB signs and symptoms (2). While the low incidence of TB in the US might constitute a barrier against this practice causing a rise in the incidence of quinolone-resistant TB, it is unclear what the effects would be in countries with medium to high incidence of TB. The high degree of cross-resistance between the old and the new generation of quinolones that we demonstrated makes it unlikely that reserving moxifloxacin for TB recommendation while using other quinolones for empiric pneumonia treatment will be beneficial in reducing the MTB moxifloxacin resistance that follows empiric treatment with a quinolone.
We found no association between moxifloxacin resistance and the Beijing family genotype, in contradistinction to data from Vietnam and Russia (9, 24). The Beijing genotype has been associated with drug resistance and MDR-TB in certain geographic locations (especially Southeast Asia, Central Asia and Eastern Europe), but not in others (7, 8, 13, 26). In the case of quinolone drug resistance, this geographic disparity in prevalence seems to apply as well. The reasons for the disparity are not clear, but epidemiologic and host factors may play a role.

A mutation in the QRDR of \textit{GyrA} was identified in only 50% of our moxifloxacin-resistant isolates. No mutations were found in the QRDR of \textit{GyrB}. In the literature, 42-85% of quinolone-resistant MTB clinical isolates harbor a mutation in the QRDR region of \textit{GyrA}, and rarely in \textit{GyrB} (12). Consistent with findings from other investigations, the isolates with the A90V mutation had a lower level of moxifloxacin resistance (MIC=1µg/ml) than isolates with the D94G or D94H mutation which were associated with higher level of resistance (MIC=4µg/ml) (1, 35). An association between MIC and a specific mutation could not be made, due to the small sample size. We did not identify a quinolone-resistance- associated mutation in the Rv2686c-Rv2687c-Rv2688c gene, which encodes a putative quinolone efflux pump. This could be due to the small number of isolates, and sequencing of this gene in a larger sample size could yield a different result. Alternatively, a different putative gene should be sequenced to account for additional mutations that are associated with quinolone resistance.

Our study has 3 important limitations. First: only 10 moxifloxacin-resistant isolates were identified. Such a small sample size limited our ability to detect resistance-associated mutations, and to examine potentially important risk factors associated with...
quinolone resistance beyond MDR-TB. Second: we did not systematically review medical records to evaluate the previous exposure to quinolones. Third, the targeted gene sequencing approach we used does not assess genome-wide all genetic loci potentially mediating quinolone-resistance. The strength of our approach lies in the comprehensive, population-based method of our sample collection which minimizes biases, and in using moxifloxacin in the susceptibility testing assay, instead of a surrogate quinolone.

In conclusion, the incidence of moxifloxacin resistance in MTB clinical isolates is low in Houston and is closely associated with MDR-TB. The issues of exposure to quinolones in the period preceding the diagnosis and the identification of mutations that are associated with quinolone resistance beyond the QRDR region of gyrA and gyrB should be further examined.
Acknowledgements

Bayer and The TB Alliance (for providing the moxifloxacin powder), Xin Ma and
Stephen Beres (The Methodist Research Institute).
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nemonoxacin (TG-873870), gemifloxacin and other quinolones against
64:428-429.


Table 1. Demographic, clinical and MTB strain characteristics of patients with TB by moxifloxacin susceptibility, Houston 2007-2008

<table>
<thead>
<tr>
<th></th>
<th>Moxifloxacin-Resistant (N=10)</th>
<th>Moxifloxacin-Susceptible (N=547)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>5</td>
<td>272</td>
<td>0.57</td>
</tr>
<tr>
<td>Mean Age (median)</td>
<td>43.6 (46)</td>
<td>44.8 (44)</td>
<td>0.83</td>
</tr>
<tr>
<td>Ethnicity/Race</td>
<td></td>
<td></td>
<td>0.58</td>
</tr>
<tr>
<td>Hispanic</td>
<td>3</td>
<td>208</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>3</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>2</td>
<td>166</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>2</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Foreign Birth</td>
<td>5</td>
<td>272</td>
<td>0.99</td>
</tr>
<tr>
<td>HIV co-infection (N=8, 433)*</td>
<td>0</td>
<td>69</td>
<td>0.22</td>
</tr>
<tr>
<td>Past TB diagnosis</td>
<td>0</td>
<td>19</td>
<td>0.56</td>
</tr>
<tr>
<td>Positive TB skin test (N=8, 324)*</td>
<td>4</td>
<td>268</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Disease site (N=10, 542)</td>
<td></td>
<td></td>
<td>0.20</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>10</td>
<td>465</td>
<td></td>
</tr>
<tr>
<td>Nonpulmonary</td>
<td>0</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>Cavitary Disease (N=10, 505)*</td>
<td>4</td>
<td>174</td>
<td>0.72</td>
</tr>
<tr>
<td>AFB sputum smear positive (N=7, 459)*</td>
<td>6</td>
<td>303</td>
<td>0.27</td>
</tr>
</tbody>
</table>
Table 1 (continued). Demographic, clinical and MTB strain characteristics of patients with TB by moxifloxacin susceptibility, Houston 2007-2008

<table>
<thead>
<tr>
<th></th>
<th>Moxifloxacin-Resistant (N=10)</th>
<th>Moxifloxacin-Susceptible (N=547)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any drug resistance (N=10, 525)*</td>
<td>3</td>
<td>65</td>
<td>0.11</td>
</tr>
<tr>
<td>Multidrug resistance (N=10, 525)*</td>
<td>3</td>
<td>5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Beijing family isolate (N=10, 544)*</td>
<td>4</td>
<td>144</td>
<td>0.34</td>
</tr>
<tr>
<td>Isolate belongs to a “PCR type” (N=10, 544)*</td>
<td>6</td>
<td>328</td>
<td>0.99</td>
</tr>
<tr>
<td>Homelessness**</td>
<td>0</td>
<td>30</td>
<td>0.45</td>
</tr>
<tr>
<td>Non-injection Illicit drug use**</td>
<td>0</td>
<td>47</td>
<td>0.33</td>
</tr>
<tr>
<td>Excess alcohol use**</td>
<td>0</td>
<td>96</td>
<td>0.15</td>
</tr>
</tbody>
</table>

* The 2 numbers in the parenthesis represent the number of patients on whom the data was available in the moxifloxacin-resistant and moxifloxacin-susceptible groups, respectively.

** Data represents the presence of homelessness, excess alcohol use and illicit drug use in the year preceding the diagnosis of TB.
Table 2. The frequency of mutations of the *GyrA*, *GyrB* in moxifloxacin-resistant and moxifloxacin-susceptible clinical MTB isolates.

<table>
<thead>
<tr>
<th></th>
<th>Moxifloxacin-resistant</th>
<th>Moxifloxacin-susceptible</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>N</em>=10</td>
<td><em>N</em>=26</td>
</tr>
<tr>
<td><em>GyrA</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A90V</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>D94H</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>D94G</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>D94A</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>G247S</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>GyrB</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G570R</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>K679R</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

All isolates (resistant and susceptible) had the following mutations: E21Q, S95T, G668D and V712L in the *GyrA* gene and P156T in the Rv2866c gene. We did not include polymorphisms that did not result in amino acid changes. The 11 new gene sequences identified in this investigation are available from NCBI GenBank, accession numbers JN012494 to JN012504.