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

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Comprehensive data on the prevalence of quinolone resistance in *Mycobacterium tuberculosis* clinical isolates in the United States are scarce. By use of a systematic population-based approach, *M. tuberculosis* strains from tuberculosis (TB) cases were collected in Harris County, TX, in 2007 to 2008. The susceptibilities of *M. tuberculosis* isolates to moxifloxacin and ofloxacin were determined by the agar proportion indirect susceptibility method. Spoligotyping and 12-locus mycobacterial interspersed repetitive unit (MIRU12)-based genotyping of *M. tuberculosis* isolates were performed, and the *gyrA*, *gyrB*, Rv2686c, Rv2687c, and Rv2688c genes in quinolone-resistant and year-of-diagnosis-matched *M. tuberculosis* isolates were sequenced. Susceptibility testing was performed on 557 *M. tuberculosis* isolates, of which 10 (1.8%) were resistant to moxifloxacin. There was 100% concordance between ofloxacin and moxifloxacin susceptibilities. A quinolone was prescribed to at least 5 (50%) patients in the period preceding TB diagnosis. Multidrug-resistant TB (MDR-TB) was significantly associated with quinolone resistance ($P = 0.01$). Mutations in the quinolone resistance-determining region of *gyrA* were found for 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 antituberculosis 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 nonmycobacterial infections, their antituberculosis 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 antituberculosis drugs. The newer quinolones, such as sparfloxacin, gatifloxacin, and moxifloxacin, have better *in vitro* activity against *Mycobacterium tuberculosis* clinical isolates than older quinolones, such as ofloxacin, levofloxacin and ciprofloxacin, as suggested by lower MICs, a higher ratio of the peak serum drug concentration to the MIC, and a higher ratio of the 24-h area under the curve to the MIC (11, 22, 27–29, 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 most favored in clinical antimycobacterial testing (4). Plans for further development of moxifloxacin as an antituberculosis agent to shorten the course of tuberculosis (TB) chemotherapy are under way, and clinicians are using the medication when there is intolerance or resistance to first-line antituberculosis agents.

However, another dynamic 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 countries where TB is endemic, quinolones can even be purchased over the counter (20). This raises the concern that *M. tuberculosis* resistance to moxifloxacin may develop for reasons 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 *M. tuberculosis* strains (17, 30, 31, 35), although a debate on whether newer quinolones retain activity in quinolone-resistant strains is ongoing (23). Two risk factors have been associated with the development of quinolone resistance in clinical *M. tuberculosis* isolates: prolonged or repeated exposure to quinolones prior to the diagnosis of TB and resistance to first-line antituberculosis drugs, especially multidrug resistance (MDR), presumably due to previous quinolone exposure (5, 6, 16, 17, 24, 35).

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

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MATERIALS AND METHODS

***M. tuberculosis* strains.** All available *M. tuberculosis* isolates recovered from patients diagnosed with TB in Houston, TX, between 1 January 2007 and 31 December 2008 were collected and sent to the Mycobacteriology Laboratory at the Texas Department of State Health Services (Texas DSHS) in Austin, TX, for quinolone susceptibility testing.

Susceptibility testing. We tested the susceptibilities of *M. tuberculosis* isolates to ofloxacin and moxifloxacin using the agar proportion indirect susceptibility assay (3). *M. tuberculosis* strains that showed ≥1% colony growth at a moxifloxacin concentration of 0.5 μg/ml or an ofloxacin concentration of 2.0 μg/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 moxifloxacin MIC for 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 the Harris County Public Health and Environmental Services.

***M. tuberculosis* molecular characterization.** All isolates were genotyped as part of the Centers for Disease Control and Prevention National Genotyping Project (http://www.cdc.gov/tb/programs/genotyping). Two genotyping methodologies were used: spoligotyping and 12-locus mycobacterial interspersed repetitive units (MIRU) (18, 32). Isolates with matching MIRU types and spoligotypes were defined as belonging to the same "PCR type."

Gene sequencing. Mutations associated with moxifloxacin and ofloxacin resistance were analyzed by sequencing PCR 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: CCTGGATG TCTAACGCAACC and AGGTACGACCGCGGGAAT, GCCGACGAAGAG GAGACC and CGTGCCTGTCCACGATTT, CGACATCGACGAGATCCAG and GCCGAGAACCTGATGGACT, and GCTGGTGAAAAAGTCCAAGC and TTCCTCCTCAGATCGCTACG. The *gyrB* gene was sequenced using the following forward and reverse primer pairs: AAACGAGGCCAGAAGATCG and CTTAACTTTGTGCGGTGCAG, CGAAACCACGGAATACGACT and GCCGAGTCACCTTCTACGAC, and CGTAAGGCACGAGAGTTGGT and GCAACGTCGTGTCTGTCATC. The Rv2686c, Rv2687c, and Rv2688c genes were sequenced using the following forward and reverse primer pairs: CTACC TGTGGCTGCGGTACT and GTTGTTGACCAGCATCATCG, CAGGCCCT GAATCTTGTTGT and CTATTCGGCCGTTATGTCGT, GTAGGTGCCTC GAATGTCGT and TGGCTGCCAAACTAACTGTG, GGCAACGAGGAAC TGAAGC and ACCACGTCGAGACCATTCAT, AACTTCTGCCGCACCT GTAG and AAAGCTCACCGGGTATGAGA, and ATCTGCATGCCCTTGG AGTA and AGACTGGTCGGAACCAGGTA. The first two genes have been widely reported in the literature to be associated with quinolone resistance in *M. tuberculosis*. The Rv2686c, Rv2687c, and Rv2688c genes putatively express an 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 by the year of diagnosis.

Human subject protection. The study was approved by the Institutional Review Boards of the Texas DSHS and the 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 ≤0.05 were considered statistically significant.

Nucleotide sequence accession numbers. The 11 new gene sequences identified in this investigation are available from GenBank under accession numbers JN012494 to JN012504.

RESULTS

In the 24-month period of the study, 634 culture-positive TB cases were reported in Houston. We performed quinolone susceptibility testing on 557 (87.8%) *M. tuberculosis* isolates. A total of 77 *M. tuberculosis* isolates did not undergo testing due to nonviability (17 isolates), contamination (22 isolates), or lack of availability (38 isolates). Resistance to moxifloxacin was found in 10 isolates (1.8%) during the study period. The moxifloxacin MIC was 1 μg/ml for 5 isolates and 4 μg/ml for 5 isolates. We found 100% concordance between resistance to moxifloxacin and resistance to ofloxacin. Although information regarding prior quinolone treatment was not always complete, we found that a quinolone was prescribed to 5 of the 10 patients with moxifloxacin-resistant isolates within 2 months prior to TB diagnosis (3 patients received moxifloxacin, 1 received ciprofloxacin, and 1 received levofloxacin). There was one documented instance of transmission of a moxifloxacin-resistant (and MDR) *M. tuberculosis* 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 ($P < 0.01$).

TABLE 1. Demographic, clinical, and *M. tuberculosis* strain characteristics of patients with TB by moxifloxacin susceptibility, Houston, Texas, 2007 to 2008

Characteristic	No. of patients[a] with:		*P*
	Moxifloxacin-resistant isolates ($n = 10$)	Moxifloxacin-susceptible isolates ($n = 547$)	
Male	5	272	0.57
Mean (median) age (yr)	43.6 (46)	44.8 (44)	0.83
Ethnicity/race			0.58
Hispanic	3	208	
White	3	76	
Black	2	166	
Asian	2	94	
Other	0	3	
Foreign birth	5	272	0.99
HIV coinfection	0/8	69/433	0.22
Past TB diagnosis	0	19	0.56
Positive TB skin test	4/8	268/324	<0.01
Disease site			0.20
Pulmonary	10	465/542	
Nonpulmonary	0	77/542	
Cavitary disease	4	174/505	0.72
AFB[b] sputum smear positive	6/7	303/459	0.27
Any drug resistance	3	65/525	0.11
Multidrug resistance	3	5/525	<0.01
Beijing family isolate	4	144/544	0.34
Isolate belongs to a "PCR type"	6	328/544	0.99
Homelessness[c]	0	30	0.45
Noninjection illicit drug use[c]	0	47	0.33
Excess alcohol use[c]	0	96	0.15

[a] In cases where data are not available for all patients in a group, the number of patients for whom the data are available is given after a slash.
[b] AFB, acid-fast bacillus.
[c] In the year preceding the diagnosis of TB.

Strain genotypes. A total of 314 PCR types were identified for the 2007–2008 *M. tuberculosis* 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

TABLE 2. Frequencies of *gyrA* and *gyrB* mutations in moxifloxacin-resistant and moxifloxacin-susceptible clinical *M. tuberculosis* isolates[a]

Mutation	No. of isolates	
	Moxifloxacin resistant (n = 10)	Moxifloxacin susceptible (n = 26)
gyrA		
A90V	2	0
D94H	1	0
D94G	1	0
D94A	1	0
G247S	1	1
gyrB		
G570R	0	1
K679R	0	1

[a] 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.

(60%) were shared with other clustered strains (range, 2 to 65 strains). The likelihood of belonging to a PCR type cluster was comparable for 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: moxifloxacin MICs were 4 μg/ml for 2 of the Beijing family strains and 1 μg/ml for 2.

Sequencing data. We identified 4 different mutations in the quinolone resistance-determining region (QRDR) of *gyrA* in 5 (50%) moxifloxacin-resistant *M. tuberculosis* isolates that were not found in the moxifloxacin-susceptible isolates. The 2 isolates with the A90V mutation had a moxifloxacin MIC of 1 μg/ml. The isolates with the D94H, D94G, and D94A mutations had moxifloxacin MICs of 4 μg/ml, 4 μg/ml, and 1 μg/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*, *gyrB*, or Rv2686c-Rv2687c-Rv2688c genes existed at a higher frequency in the quinolone-resistant isolates than in the susceptible isolates (Table 2).

DISCUSSION

Using a population-based approach, we determined the moxifloxacin susceptibilities of 87.8% of the *M. tuberculosis* strains isolated over a 24-month period in Houston, TX. 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 *M. tuberculosis* 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 as much as 28% of all new cases, according to World Health Organization statistics (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 in the period preceding TB diagnosis was common among patients with quinolone-resistant TB. The empirical treatment of pneumonia patients with quinolones is a common and recommended practice, which may mask some TB signs and symptoms (2). While the low incidence of TB in the United States might constitute a barrier against this practice causing a rise in the incidence of quinolone-resistant TB, it is not clear what the effects would be in countries with medium to high incidences of TB. The high degree of cross-resistance between older and newer quinolones that we demonstrated makes it unlikely that reserving moxifloxacin for TB recommendation while using other quinolones for empirical pneumonia treatment will be beneficial in reducing the *M. tuberculosis* moxifloxacin resistance that follows empirical 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, 12, 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 *gyrA* was identified in only 50% of our moxifloxacin-resistant isolates. No mutations were found in the QRDR of *gyrB*. In the literature, 42 to 85% of quinolone-resistant *M. tuberculosis* clinical isolates harbor a mutation in the QRDR region of *gyrA*, but such isolates rarely harbor a mutation in the *gyrB* QRDR (13). Consistent with findings from other investigations, isolates with the A90V mutation had a lower level of moxifloxacin resistance (MIC, 1 μg/ml) than isolates with the D94G or D94H mutation (MIC, 4 μg/ml) (1, 35). No association could be made between the MIC and a specific mutation, 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; sequencing of this gene in a larger sample 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 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 the use of moxifloxacin, instead of a surrogate quinolone, in the susceptibility testing assay.

In conclusion, the incidence of moxifloxacin resistance in *M. tuberculosis* 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 QRDRs of *gyrA* and *gyrB* should be further examined.

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