Moxifloxacin Improves Treatment Outcomes in Patients with Ofloxacin-Resistant Multidrug-Resistant Tuberculosis

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It is unclear whether the use of moxifloxacin (MFX), a newer synthetic fluoroquinolone, results in better outcomes in patients with ofloxacin (OFX)-resistant multidrug-resistant tuberculosis (MDR-TB). During the period from April 2006 to December 2013, a total of 2,511 patients with culture-confirmed tuberculosis (TB) were treated at a TB referral hospital in southern Taiwan. Of the 2,511 patients, 325 (12.9%) had MDR-TB, and of those 325 patients, 81 (24.9%) had OFX-resistant MDR-TB and were included in the study. Among the 81 patients with OFX-resistant MDR-TB, 50 (61.7%) were successfully treated and 31 (38.3%) had unfavorable outcomes, including treatment failure ($n = 25$; 30.9%), loss to follow-up ($n = 2$; 2.5%), and death ($n = 4$; 4.9%). Patients treated with MFX had a significantly higher rate of treatment success (77.3% versus 43.2%; odds ratio [OR] = 4.46, 95% confidence interval [CI] = 1.710 to 11.646, $P = 0.002$) than patients not treated with MFX, especially among those infected with MFX-susceptible isolates (40.7%) or isolates with low-level resistance to MFX (28.4%). Multivariate logistic regression analysis showed that treatment with MFX (adjusted odds ratio = 6.54, 95% CI = 1.44 to 29.59, $P = 0.015$) was the only independent factor associated with treatment success. Mutation at codon 94 in the *gyrA* gene was the most frequent mutation (68.0%) associated with high-level MFX resistance. Multivariate Cox proportional hazards regression analysis showed that treatment with MFX was also an independent factor associated with early culture conversion (hazard ratio = 3.12, 95% CI = 1.48 to 6.54, $P = 0.003$). Our results show that a significant proportion of OFX-resistant MDR-TB isolates were susceptible or had low-level resistance to MFX, indicating that patients with OFX-resistant MDR-TB benefit from treatment with MFX.

Fluoroquinolones are the most important agents for the treatment of multidrug-resistant tuberculosis (MDR-TB), which is defined as TB caused by bacteria that are resistant to both of the most effective antituberculosis drugs, isoniazid and rifampin (1, 2). However, the emergence of ofloxacin (OFX)-resistant MDR-TB and extensively drug-resistant TB (XDR-TB) (3, 4), defined as further resistance to any fluoroquinolone and second-line injectable drug, has further complicated patient care.

Moxifloxacin (MFX), a newer fluoroquinolone, has been shown to have better activity against *Mycobacterium tuberculosis* than OFX (5, 6) and to have incomplete cross-resistance to OFX (7). MFX has lower MICs than older fluoroquinolones, such as OFX or ciprofloxacin, and therefore may be an effective agent against OFX-resistant MDR-TB (8, 9). Studies have shown that MFX is effective against clinical isolates of OFX-resistant *M. tuberculosis* *in vitro* (9) and XDR-TB in mice (10). However, it is still unclear whether the use of MFX offers any clinical benefit in patients with OFX-resistant MDR-TB or XDR-TB.

WHO guidelines suggest that drug susceptibility testing for MFX should involve measurements at two critical concentrations, namely, 0.5 mg/liter (low-level resistance) and 2.0 mg/liter (high-level resistance) (11). However, no clinical studies have evaluated how the levels of MFX resistance influence the outcomes of patients who receive MFX for the treatment of OFX-resistant MDR-TB. In this study, we investigated the treatment outcomes of patients with OFX-resistant MDR-TB and, in particular, how the use of MFX affected these outcomes.

MATERIALS AND METHODS

Hospital setting and patients. This study was performed in a TB referral hospital in southern Taiwan. Data on all patients treated for TB during the period from April 2006 to December 2013 were reviewed to identify pa-

tients with OFX-resistant MDR-TB. In Taiwan, all patients with TB are registered in the national TB registry database maintained by the Taiwan Centers for Disease Control (Taiwan CDC). In addition, all patients in Taiwan with MDR-TB are managed according to the Directly Observed Therapy Strategy (DOTS) program. The protocol includes monitoring of patients twice daily for adherence and adverse events, the monthly submission of sputum samples for mycobacterial culture, and the continuation of medication for 18 months after conversion of a sputum culture from positive to negative. All patients in this study were treated with individualized regimens that were based on their drug susceptibility test results and treatment history. Treatment regimens included at least four antituberculosis drugs likely to be effective in the intensive phase, as recommended by the WHO (1). This study was approved by the Institutional Review Board of the National Taiwan University Hospital (201506046RINB).

Laboratory study. Sputum samples were decontaminated and liquefied using NaOH and concentrated by centrifugation. The smears were stained using the Ziehl-Neelsen technique and examined under a high-power microscope. Processed samples were inoculated into two types of media: Bactec MGIT liquid medium (Bactec *Mycobacterium* growth indicator tube; MGIT 960 system; Becton Dickinson) and Lowenstein-Jensen solid medium. Mycobacterial isolates were identified to the species level using conventional biochemical testing, and susceptibility testing was per-

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TABLE 1 Characteristics of patients with OFX-resistant MDR-TB

Characteristic	No. (%) of patients			P value
	All	Treatment success	Unfavorable outcomes	
Total	81	50	31	
Age (yr)				0.456
<45	21 (25.9)	15 (30.0)	6 (19.4)	
45–64	48 (59.3)	27 (54.0)	21 (67.7)	
≥65	12 (14.8)	8 (16.0)	4 (12.9)	
Male	67 (82.7)	40 (80.0)	27 (87.1)	0.550
Prior treatment of TB	25 (30.9)	9 (18.0)	16 (51.6)	0.001
Comorbidity				
Diabetes mellitus	29 (35.8)	15 (30.0)	14 (45.2)	0.167
Hypertension	17 (21.0)	10 (20.0)	7 (22.6)	0.782
Liver cirrhosis	5 (6.2)	2 (4.0)	3 (9.7)	0.366
Smear positive	66 (81.5)	37 (74.0)	29 (93.5)	0.038
Findings of CXR ^a				
Disease extent				0.025
Mild	12 (14.8)	11 (22.0)	1 (3.2)	
Moderate/severe	69 (85.2)	39 (78.0)	30 (96.8)	
Lower lung involvement	50 (61.7)	29 (58.0)	21 (67.7)	0.381
Cavitary lesions	49 (60.5)	28 (56.0)	21 (67.7)	0.293
Cavity size				0.401
<4 cm	29 (35.8)	18 (36.0)	11 (35.5)	
≥4 cm	20 (24.7)	10 (20.0)	10 (32.3)	
Pleural effusion	14 (17.3)	8 (16.0)	6 (19.4)	0.698
Drug resistance				
Ethambutol resistance	30 (37.0)	17 (34.0)	13 (41.9)	0.472
Streptomycin resistance	42 (51.9)	29 (58.0)	13 (41.9)	0.160
Kanamycin resistance	26 (32.1)	14 (28.0)	12 (38.7)	0.316
Moxifloxacin-containing regimens	44 (54.3)	34 (68.0)	10 (32.3)	0.002
Drugs throughout the initiation phase				
Second-line injectable agents	36 (44.4)	29 (58.0)	7 (22.6)	0.002
Ethambutol	51 (63.0)	33 (66.0)	18 (58.1)	0.472
Prothionamide	77 (95.1)	46 (92.0)	31 (100.0)	0.106
Cycloserine	63 (77.8)	36 (72.0)	27 (87.1)	0.112
<i>para</i> -Aminosalicylic acid	65 (80.2)	42 (84.0)	23 (74.2)	0.281
Pyrazinamide	34 (42.0)	19 (38.0)	15 (48.4)	0.357
Linezolid	2 (2.5)	1 (3.2)	1 (2.0)	1.000
No. of likely effective drugs				
≥4	81 (100.0)	50 (100.0)	31 (100.0)	1.000
≥5	52 (64.2)	35 (70.0)	17 (54.8)	0.167
≥6	29 (35.8)	19 (38.0)	10 (32.3)	0.600
Surgery	5 (6.2)	5 (10.0)	0 (0.0)	0.151
Moxifloxacin resistance				0.175
Susceptible (MIC ≤ 0.5 mg/liter)	33 (40.7)	21 (42.0)	12 (38.7)	
Low level (MIC = 1.0–2.0 mg/liter)	23 (28.4)	17 (34.0)	6 (19.4)	
High level (MIC > 2.0 mg/liter)	25 (30.9)	12 (24.0)	13 (41.9)	
Gene mutations ^b				
<i>gyrA</i>	67 (82.7)	38 (76.0)	27 (87.1)	0.264
G88C (gGC/tGC)	1 (1.2)	1 (2.0)	0 (0.0)	1.000
A90V (GcG/GtG)	5 (6.2)	3 (6.0)	2 (6.5)	1.000
S91P (tCG/cCG)	2 (2.5)	0 (0.0)	2 (6.5)	0.144

(Continued on following page)

TABLE 1 (Continued)

Characteristic	No. (%) of patients			P value
	All	Treatment success	Unfavorable outcomes	
Codon 94	26 (32.1)	9 (18.0)	17 (54.8)	0.001
D94G (gaC/ggC)	11 (13.6)	2 (4.0)	9 (29.0)	
D94N (gaC/aaC)	7 (8.6)	4 (8.0)	3 (9.7)	
D94A (gaC/gcC)	5 (6.2)	3 (6.0)	2 (6.5)	
D94H (gaC/caC)	1 (1.2)	0 (0.0)	1 (3.2)	
D94Y (gaC/taC)	2 (2.5)	0 (0.0)	2 (6.5)	
A132S (gCC/tCC)	2 (2.5)	1 (2.0)	1 (3.2)	1.000
P190L (CcG/CtG)	1 (1.2)	0 (0.0)	1 (3.2)	0.383
A288D (GcC/GaC)	1 (1.2)	0 (0.0)	1 (3.2)	0.383
A384V (GcA/GtA)	12 (14.8)	9 (18.0)	3 (9.7)	0.356
<i>gyrB</i>	25 (30.9)	16 (32.0)	7 (22.6)	0.451
H244N (cAC/aAC)	1 (1.2)	0 (0.0)	1 (3.2)	0.383
M291I (ATg/ATc)	12 (14.8)	9 (18.0)	3 (9.7)	0.356
A403S (gCG/tCG)	3 (3.7)	2 (4.0)	1 (3.2)	1.000
D461N (gAC/aAC)	1 (1.2)	1 (2.0)	0 (0.0)	1.000
I485V (aTC/gTC)	1 (1.2)	1 (2.0)	0 (0.0)	1.000
Any codon 499	2 (2.5)	1 (2.0)	1 (3.2)	1.000
N499D (AAc/GAc)	1 (1.2)	0 (0.0)	1 (3.2)	0.383
N499T (AAc/ACc)	1 (1.2)	1 (2.0)	0 (0.0)	1.000
A504T (gCG/aCG)	1 (1.2)	0 (0.0)	1 (3.2)	0.383
A508S (gCG/tCG)	1 (1.2)	1 (2.0)	0 (0.0)	1.000
G512R (gGG/aGG)	3 (3.7)	2 (4.0)	1 (3.2)	1.000
Wild type	13 (16.0)	10 (20.0)	3 (9.7)	0.351

^a CXR, chest X ray.^b Lowercase letters indicate the mutated nucleotides.

formed using the indirect agar proportion method (12). The critical concentrations were 0.2 µg/ml for isoniazid, 1 µg/ml for rifampin, 5 µg/ml for ethambutol, 2 µg/ml for streptomycin, 6 µg/ml for kanamycin, and 2 µg/ml for OFX. The MIC was measured with a Sensititre MycoTB plate (Trek Diagnostic Systems, Cleveland, OH, USA) as previously described (13), and the treating clinicians were blind to the results. Susceptibility to MFX was defined as a MIC level of ≤0.5 µg/ml, low-level resistance was defined as a MIC level ranging from 1.0 to 2.0 µg/ml, and high-level resistance was defined as a MIC level of >2 µg/ml (14). All patients were tested for antibodies to human immunodeficiency virus (anti-HIV; Abbott AxSYM HIV assay; Abbott Laboratories, Abbott Park, IL, USA).

DNA sequencing. The *gyrA* and *gyrB* genes were amplified and sequenced with 8 primer sets. For *gyrA*, the primer sets were GYRA1 (forward primer, 5'-GAT GTC TAA CGC AAC CCT GCG TTC GAT; reverse primer, 5'-AGG TAC GAC CGC GGG AAT CCT CTT CTA), GYRA2 (forward primer, 5'-CCG ACG CGG TGT TCT GG; reverse primer, 5'-TCG ATT TTG GCC AGG TCG TC), GYRA3 (forward primer, 5'-GTC GGA GAC CGT CGA TA; reverse primer, 5'-GAA CCT GAT GGA CTG CCC), and GYRA4 (forward primer, 5'-GCA ACG GGC TGG TGA AAA AG; reverse primer, 5'-GAC CAA GCC ATC CGC ATT C). For the *gyrB* gene, the primer sets were GYRB1 (forward primer, 5'-ATC GCC GCA GCG GTT G; reverse primer, 5'-ACC TGA GAC CAC TCG TAC CC), GYRB2 (forward primer, 5'-TCG ACG TGG TGA TGA CAC AA; reverse primer, 5'-GCT GAC CTT CAC CGA GAT CA), GYRB3 (forward primer, 5'-CGG CAC CCA CGA AGA GG; reverse primer, 5'-ATG CGA ATT CCG GGT CAC TG), and GYRB4 (forward primer, 5'-GAG TTC GAT ATC GGC AAG CTG; reverse primer, 5'-CCC GTC GCG CAC CTC). The sequences were assembled and compared to the H37Rv reference strain sequence (GenBank accession number NC_000962.3).

Data collection. The patients' medical records were reviewed to obtain data on associated medical conditions, the status of cultures/smears for acid-fast bacilli during treatment, the treatment course, and outcomes. We ascertained the treatment history using a self-report questionnaire and by cross-checking the answers with data in the TB registry database.

Baseline chest X rays (CXRs) before treatment were evaluated by an experienced pulmonologist who was blind to the clinical data. The extent of disease on the baseline CXR was evaluated as a radiographic score (15). Briefly, each lung was divided into 3 areas, and the extent of infiltration in each area was rated on a four-point scale ranging from 0 to 3, with the maximum radiographic score being 18. Higher scores indicated a greater extent of disease involvement. Mild disease was defined in patients with radiographic scores ranging from 0 to 6, moderate disease was defined in patients with scores ranging from 7 to 12, and severe disease was defined in patients with scores ranging from 13 to 18.

The treatment course was reviewed and defined as two phases, namely, an intensive phase of 8 months and a continuation phase (16). Drug use was defined as the use of a drug for at least 80% of the time during the defined treatment period. The six treatment outcome categories used in this study were based on those recommended by the WHO for patients with MDR-TB/XDR-TB who receive second-line treatment: cure, completion of treatment, treatment failure, death, loss to follow-up, and no evaluation (16). Treatment failure was defined in patients whose treatment was terminated and in those who required a change in the permanent regimen involving at least two antituberculosis drugs because of (i) a lack of conversion by the end of the intensive phase, (ii) bacteriological reversion in the continuation phase, (iii) additional acquired resistance to fluoroquinolones or second-line injectable drugs, or (iv) adverse drug reactions. The cured and completed treatment categories were defined as treatment success, whereas treatment failure, death, and loss to follow-up were defined as unfavorable outcomes. Culture conversion (to negative) was defined as negative results for two consecutive cultures of samples taken at least 30 days apart. The date of culture conversion was defined as the date of the first negative culture result. Culture status was considered to have reverted to positive when, after an initial conversion, two consecutive cultures of samples taken at least 30 days apart were found to be positive.

Statistical analysis. Categorical variables were compared using the chi-square test or Fisher's exact test, where appropriate, and differences in

continuous variables were analyzed using the Mann-Whitney U test. Data are presented as the number (percent) of patients or mean $\pm$ standard deviation unless otherwise noted. A variance inflation factor was used to quantify the severity of multicollinearity, and only variables with *P* values of <0.05 in the univariate analysis and a variance inflation factor of <4 were included in the multivariate analysis. Multivariate logistic regression analysis with the forced-in method was used to determine independent variables that were predictive of treatment success. Kaplan-Meier time-to-event curves for culture conversion were constructed, and subgroups were compared using the log rank test. Multivariate Cox proportional hazards regression with the forced-in method was used to determine independent variables that were predictive of culture conversion. A *P* value of <0.05 was considered to indicate statistical significance; all tests were two-tailed. All statistical analyses were performed using the statistical package SPSS for Windows (version 17; SPSS, Chicago, IL, USA).

RESULTS

From April 2006 to December 2013, a total of 2,511 patients with culture-confirmed TB were treated at our hospital, and 325 (12.9%) had MDR-TB. Of those 325 patients, 81 (24.9%) patients were identified to have OFX-resistant MDR-TB, including 26 (32.1%) with XDR-TB. All subjects had negative serological test results for HIV infection. The majority of patients with OFX-resistant MDR-TB were men ($n = 67$, 82.7%), and the mean age was 51.2 ± 13.5 years. Among the 81 patients, 44 (54.3%) were treated with MFX and 36 (44.4%) received second-line injectable agents, including kanamycin ($n = 30$, 83.3%) and capreomycin ($n = 6$, 16.7%).

Of the 81 patients with OFX-resistant MDR-TB, 50 (61.7%) had treatment success (all cured) and 31 (38.3%) had unfavorable outcomes, including 25 (30.9%) who had treatment failures, 2 (2.5%) who were lost to follow-up, and 4 (4.9%) who died (Table 1). There were no significant differences among the patients in age, gender, number of comorbidities, disease extent as measured radiographically, or susceptibility to ethambutol, streptomycin, or kanamycin. However, the percentages of patients with a history of antituberculosis treatment (51.6% versus 18.0%, $P = 0.001$) and smear-positive TB (93.5% versus 74.0%, $P = 0.038$) were significantly higher among those in the unfavorable outcome group than among patients in the treatment success group. Furthermore, the percentages of patients treated with MFX (32.3% versus 68.0%, $P = 0.002$) and second-line injectable agents (22.6% versus 58.0%, $P = 0.002$) were significantly lower in the unfavorable outcome group than the treatment success group.

As shown in Table 2, there were no significant differences in demographic characteristics, smear results, or radiographic findings between patients treated with MFX and those who did not receive MFX. However, patients treated with MFX were more likely than patients who did not receive MFX to be treated with second-line injectable agents (61.4% versus 24.3%, $P = 0.001$), more likely to have MFX-susceptible or low-level MFX-resistant MDR-TB ($P = 0.026$), more likely to have a higher treatment success rate (77.3% versus 43.2%, odds ratio [OR] = 4.46, 95% confidence interval [CI] = 1.71 to 11.65, $P = 0.002$), and more likely to have higher culture conversion rates at the 6th (75.0% versus 43.2%) and 12th (79.5% versus 48.6%) months of treatment. Variables that were identified by the univariate analysis, including history of anti-TB treatment (adjusted odds ratio [AOR] = 0.26, 95% CI = 0.06 to 1.07, $P = 0.061$), smear-positive TB (AOR = 0.47, 95% CI = 0.06 to 3.42, $P = 0.454$), moderate to severe disease extent (AOR = 0.13, 95% CI = 0.01 to 1.58, $P =$

TABLE 2 Characteristics of patients with OFX-resistant MDR-TB treated with or without MFX-containing regimens

Characteristic	No. (%) of patients receiving MFX-containing regimens or not		<i>P</i> value
	Yes	No	
Total	44	37	
Age (yr)			0.629
<45	13 (29.5)	8 (21.6)	
45–64	24 (54.5)	24 (64.9)	
≥ 65	7 (15.9)	5 (13.5)	
Male	38 (86.4)	29 (78.4)	0.344
Prior treatment of TB	11 (25.0)	14 (37.8)	0.213
Comorbidity			
Diabetes mellitus	13 (29.5)	16 (43.2)	0.200
Hypertension	8 (18.2)	9 (24.3)	0.499
Liver cirrhosis	2 (4.5)	3 (8.1)	0.656
Smear positive	33 (75.0)	33 (89.2)	0.151
Findings of CXR ^a			0.268
Disease extent			
Mild	9 (20.5)	3 (8.1)	
Moderate	24 (54.5)	25 (67.6)	
Severe	11 (25.0)	9 (24.3)	
Lower lung involvement	26 (59.1)	24 (64.9)	0.550
Cavitary lesions	25 (56.8)	24 (64.9)	0.461
Cavity size			0.296
<4 cm	13 (29.5)	16 (43.2)	
≥ 4 cm	12 (27.3)	8 (21.6)	
Pleural effusion	7 (15.9)	7 (18.9)	0.721
Drug resistance			
Ethambutol resistance	15 (34.1)	15 (40.5)	0.549
Streptomycin resistance	26 (59.1)	16 (43.2)	0.155
Kanamycin resistance	13 (29.5)	13 (35.1)	0.591
Drugs throughout the initiation phase			
Moxifloxacin	44 (100.0)	0 (0.0)	0.001
Second-line injectable agents	27 (61.4)	9 (24.3)	0.001
Linezolid	1 (2.3)	1 (2.7)	0.901
No. of likely effective drugs			
≥ 4	44 (100.0)	37 (100.0)	1.000
≥ 5	27 (61.4)	25 (67.6)	0.562
≥ 6	15 (34.1)	14 (37.8)	0.726
Moxifloxacin resistant			0.026
Susceptible (MIC ≤ 0.5 μ g/ml)	21 (47.7)	12 (32.4)	
Low level (MIC = 1.0–2.0 μ g/ml)	15 (34.1)	8 (21.6)	
High level (MIC > 2.0 μ g/ml)	8 (18.2)	17 (45.9)	
Surgery	3 (6.8)	2 (5.4)	1.000
Culture conversion			
6th mo	33 (75.0)	16 (43.2)	0.006
12th mo	35 (79.5)	18 (48.6)	0.005
24th mo	37 (84.1)	28 (75.7)	0.676
Outcomes			0.002
Treatment success	34 (77.3)	16 (43.2)	
Unfavorable outcomes	10 (22.7)	21 (56.8)	
Death	1 (2.3)	3 (8.1)	
Loss to follow-up	0 (0.0)	2 (5.4)	
Treatment failure	9 (20.5)	16 (43.2)	

^a CXR, chest X ray.

FIG 1 Kaplan-Meier plots and log rank test for probability of culture conversion. The Kaplan-Meier time-to-event analysis is based on treatment with or without MFX, stratified by levels of moxifloxacin resistance.

0.110), treatment with MFX (AOR = 6.54, 95% CI = 1.44 to 29.59, $P = 0.015$), treatment with second-line injectable agents (AOR = 2.18, 95% CI = 0.59 to 8.09, $P = 0.245$), and high-level resistance of MFX (AOR = 1.41, 95% CI = 0.30 to 6.70, $P = 0.666$), and an interaction term involving susceptibility to MFX and treatment with MFX ($P = 0.013$) were introduced into the multivariate logistic regression analysis and showed that treatment with MFX was the only independent factor associated with treatment success.

There was no significant difference in the treatment success rate among patients with high-level MFX-resistant MDR-TB, those with MFX-susceptible MDR-TB, and patients with low-level MFX-resistant MDR-TB (48.0% [12/25], 63.6% [21/33], and 73.9% [17/23], respectively; $P = 0.175$). Among patients with MFX-susceptible MDR-TB and those with low-level MFX-resistant MDR-TB, treatment with MFX was associated with a higher

treatment success rate than treatment without MFX (success rates for patients with MFX-susceptible MDR-TB treated and not treated with MFX, 85.7% [18/21] versus 25% [3/12], respectively [OR = 18.0, 95% CI = 2.36 to 156.52, $P < 0.001$]; success rates for patients with low-level MFX-resistant MDR-TB treated and not treated with MFX, 86.7% [13/15] versus 50% [4/8], respectively [OR = 6.5, 95% CI = 0.59 to 89.24, $P = 0.057$]). However, there was no significant difference in the rate of treatment success among patients with high-level MFX-resistant MDR-TB who received MFX and those who did not (37.5% [3/8] versus 52.9% [9/17], respectively; OR = 0.53, 95% CI = 0.06 to 3.94, $P = 0.471$). The Kaplan-Meier time-to-event analysis revealed that patients with OFX-resistant MDR-TB who were treated with MFX had culture conversion significantly sooner than those who did not receive MFX (median time to culture conversion, 74 versus 362 days, respectively; $P = 0.004$ by log-rank test; Fig. 1A), espe-

TABLE 3 Mutations in *gyrA* and *gyrB* genes and level of MFX resistance in OFX-resistant MDR-TB

Characteristic	No. (%) of isolates with the following MFX resistance:			P value
	Susceptible (MIC $\leq$ 0.5 μ g/ml)	Low level (MIC = 1.0–2.0 μ g/ml)	High level (MIC $>$ 2.0 μ g/ml)	
Total	33	23	25	
Gene mutations ^a				
<i>gyrA</i>	26 (78.8)	18 (78.3)	23 (92.0)	0.322
G88C (gGC/tGC)	0 (0.0)	0 (0.0)	1 (4.0)	0.593
A90V (GcG/GtG)	4 (12.1)	1 (4.3)	0 (0.0)	0.177
S91P (tCG/cCG)	0 (0.0)	2 (8.7)	0 (0.0)	0.078
Codon 94	4 (12.1)	5 (21.7)	17 (68.0)	<0.001
D94G (gaC/ggC)	2 (6.1)	1 (4.3)	8 (32.0)	
D94N (gaC/aaC)	1 (3.0)	0 (0.0)	6 (24.0)	
D94A (gaC/gcC)	0 (0.0)	3 (13.0)	2 (8.0)	
D94H (gaC/caC)	1 (3.0)	0 (0.0)	0 (0.0)	
D94Y (gaC/taC)	0 (0.0)	1 (4.3)	1 (4.0)	
A132S (gCC/tCC)	0 (0.0)	0 (0.0)	2 (8.0)	0.171
P190L (CcG/CtG)	0 (0.0)	0 (0.0)	1 (4.0)	0.593
A288D (GcC/GaC)	1 (3.0)	0 (0.0)	0 (0.0)	1.000
A384V (GcA/GtA)	6 (18.2)	2 (8.7)	4 (16.0)	0.673
<i>gyrB</i>	8 (24.2)	7 (30.4)	10 (40.0)	0.436
H244N (cAC/aAC)	0 (0.0)	0 (0.0)	1 (4.0)	0.593
M291I (ATg/ATc)	7 (21.2)	1 (4.3)	4 (16.0)	0.234
A403S (gCG/tCG)	0 (0.0)	3 (13.0)	0 (0.0)	0.021
D461N (gAC/aAC)	1 (3.0)	0 (0.0)	0 (0.0)	1.000
I485V (aTC/gTC)	0 (0.0)	1 (4.3)	0 (0.0)	0.284
Any codon 499	0 (0.0)	0 (0.0)	2 (8.0)	0.171
N499D (aaC/gaC)	0 (0.0)	0 (0.0)	1 (4.0)	
N499T (aaC/acC)	0 (0.0)	0 (0.0)	1 (4.0)	
A504T (gCG/aCG)	0 (0.0)	0 (0.0)	1 (4.0)	0.593
A508S (gCG/tCG)	0 (0.0)	1 (4.3)	0 (0.0)	0.284
G512R (gGG/aGG)	0 (0.0)	0 (0.0)	3 (12.0)	0.048
Wild type	6 (18.2)	5 (21.7)	2 (8.0)	0.410

^a Lowercase letters indicate the mutated nucleotides.

cially among those with MFX-susceptible MDR-TB (MIC $\leq$ 0.5 μ g/ml; $P = 0.033$; Fig. 1B) and low-level MFX-resistant MDR-TB (MIC = 1.0 to 2.0 μ g/ml; $P = 0.003$; Fig. 1C); however, there was no significant difference in the time to culture conversion among those with high-level MFX-resistant MDR-TB (MIC $>$ 2.0 μ g/ml; $P = 0.640$; Fig. 1D).

As seen in Table 3, mutations in either the *gyrA* or *gyrB* gene were found in 81.2% (27/33) of MFX-susceptible isolates, 78.3% (18/23) of low-level MFX-resistant isolates, and 92.0% (23/25) of high-level MFX-resistant *M. tuberculosis* isolates. Of the 26 *M. tuberculosis* isolates with mutations at codon 94 of the *gyrA* gene, 17 (65.4%) had high-level resistance to MFX. As seen in Fig. 2, the four subgroups of patients, stratified by treatment with MFX and the presence of mutations at codon 94 of *gyrA*, were significantly different by the time to culture conversion ($P = 0.001$). Treatment with MFX tended to correlate with earlier culture conversion among patients whose isolates had mutations at codon 94 of *gyrA*.

Variables that were identified by the univariate analysis to be associated with culture conversion were included in a multivariate Cox proportional hazards model, including an interaction term involving MFX resistance (high-level resistance versus susceptible or low-level resistance) and MFX use (Table 4). The results showed that treatment with an MFX-containing regimen was an independent factor associated with earlier culture conversion (hazard ratio [HR] = 3.12, 95% CI = 1.48 to 6.54, $P = 0.003$) and that smear

positivity at the initiation of treatment was significantly associated with a delayed culture conversion (HR = 0.42, 95% CI = 0.21 to 0.86, $P = 0.017$), especially among those with MFX-susceptible and low-level MFX-resistant MDR-TB.

FIG 2 Kaplan-Meier plots and log rank test for probability of culture conversion. The Kaplan-Meier time-to-event analysis is based on treatment with or without MFX and the presence of mutations at codon 94 in the *gyrA* gene.

TABLE 4 Factors predicting culture conversion among patients with OFX-resistant MDR-TB^a

Characteristic	Univariate analysis			Multivariate analysis		
	HR	95% CI	P value	HR	95% CI	P value
Age (yr)						
<45	1.00					
45–64	1.88	0.97–3.64	0.060			
≥65	1.59	0.89–2.83	0.116			
Male	0.87	0.47–1.63	0.667			
Prior treatment of TB	0.49	0.28–0.85	0.012	0.55	0.28–1.09	0.086
Comorbidity						
Diabetes mellitus	0.66	0.40–1.10	0.114			
Hypertension	1.17	0.66–2.06	0.585			
Liver cirrhosis	1.17	0.47–2.93	0.735			
Smear positive	0.36	0.20–0.66	0.001	0.42	0.21–0.86	0.017
Findings of CXR						
Disease extent						
Mild	1.00					
Moderate/severe	0.39	0.21–0.76	0.005	0.68	0.32–1.41	0.299
Lower lung involvement	0.86	0.53–1.40	0.544			
Cavitary lesions	0.67	0.41–1.10	0.112			
Pleural effusion	0.96	0.53–1.95	1.018			
Drug resistance						
Ethambutol resistance	1.13	0.68–1.86	0.645			
Streptomycin resistance	1.21	0.75–1.97	0.437			
Kanamycin resistance	0.94	0.56–1.57	0.803			
Moxifloxacin-containing regimens	2.22	1.33–3.71	0.002	3.12	1.48–6.54	0.003
Drugs throughout the initiation phase						
Second-line injectable agents	1.93	1.17–3.18	0.010	1.12	0.59–2.10	0.737
Ethambutol	0.89	0.54–1.47	0.645			
Prothionamide	0.48	0.17–1.35	0.163			
Cycloserine	0.64	0.36–1.12	0.116			
<i>para</i> -Aminosalicylic acid	1.32	0.70–2.47	0.390			
Pyrazinamide	0.80	0.48–1.31	0.365			
Linezolid	0.95	0.23–3.91	0.944			
No. of likely effective drugs						
≥5	1.07	0.64–1.78	0.806			
≥6	0.81	0.49–1.34	0.404			
Moxifloxacin resistance						
Susceptible/low level	1.00					
High level	0.68	0.40–1.14	0.142	1.33	0.60–2.97	0.481
Interaction ^b				0.32	0.10–1.01	0.052

^a CI, confidence interval; CXR, chest X ray; HR, hazard ratio.^b Interaction term involving high-level moxifloxacin resistance and moxifloxacin-containing regimens.

DISCUSSION

Our study showed that the rate of treatment success was significantly greater among patients with OFX-resistant MDR-TB who were treated with MFX. We also found that an MFX-containing regimen was independently associated with earlier culture conversion in patients with MFX-susceptible MDR-TB and in those with low-level MFX-resistant MDR-TB.

A meta-analysis revealed that treatment with newer fluoroquinolones, including MFX, improves treatment outcomes in patients with XDR-TB, even when drug susceptibility testing

demonstrates resistance to a representative fluoroquinolone (17). Recently, Jo et al. found that patients with OFX-resistant MDR-TB had significantly better treatment outcomes when isolates were susceptible to MFX (18). They speculated that this was due to the effect of newer fluoroquinolones, although no treatment data were provided to support their speculation. Sirgel et al. (9) found that for *M. tuberculosis* strains with mutations in the quinolone resistance-determining region (QRDR) of the *gyrA* gene, the OFX MIC₅₀ was 8.0 µg/ml and the MIC₉₀ was >10 µg/ml, whereas the MFX MIC values were ≤2.0 µg/ml in 96% of

the strains, which is within the achievable peak serum concentration of 4 mg/liter with daily oral dosing of 400 mg (19). Kambli et al. (20) found that MFX has significantly better pharmacokinetic and pharmacodynamic effects than OFX even against isolates with resistance-associated mutations in the *gyrA* gene. We found that patients with TB due to *M. tuberculosis* strains with low-level resistance to MFX (MICs ≤ 2.0 $\mu\text{g/ml}$) had better treatment outcomes and earlier culture conversion. Thus, it is conceivable that low-level resistance to MFX might not correspond to clinical resistance (21). The recommended epidemiological cutoff (ECOFF) which differentiates wild-type from non-wild-type strains for MFX resistance in Middlebrook 7H10 medium is 0.5 $\mu\text{g/ml}$; however, the most recent WHO guidelines suggest that MFX resistance be tested at two critical concentrations: 0.5 $\mu\text{g/ml}$ (for low-level resistance) and 2.0 $\mu\text{g/ml}$ (for high-level resistance) (22). Our findings support that recommendation. In addition, we found that an MFX-based regimen is clinically beneficial for patients with TB due to OFX-resistant multidrug-resistant pathogens.

Studies have shown that differences in mutations in the *gyrA* and *gyrB* genes correlate with different levels of fluoroquinolone resistance (21). For example, studies have demonstrated that the majority of OFX-resistant *M. tuberculosis* strains harboring the G88A, A90V, A94A, and D94G mutations in the *gyrA* gene (9, 18) and the D500A or N538T mutation in the *gyrB* gene are susceptible to MFX (23). Kambli et al. (20) recently reported that MFX MIC values were consistently lower than OFX MIC values among *M. tuberculosis* isolates with the same *gyrA* mutation. Although the majority of *M. tuberculosis* isolates harboring mutations in codon 94 of *gyrA* in their study had a moderate level of resistance to MFX (MICs, 2.5 $\mu\text{g/ml}$), they also found 3 isolates with mutations at codon 94 with a high level of resistance to MFX (MICs, 5.0 to 8.0 $\mu\text{g/ml}$). We found that mutations at codon 94 in the *gyrA* gene were the most frequent mutations associated with high-level MFX resistance. Among 26 *M. tuberculosis* strains with mutations at codon 94, 17 (65.4%) strains had MFX MICs of >2.0 $\mu\text{g/ml}$. Interestingly, however, the addition of MFX to treatment regimens still tended to facilitate culture conversion in patients with TB due to *M. tuberculosis* strains harboring mutations at codon 94 of the *gyrA* gene (Fig. 2).

The activity of MFX correlates with its MIC values, and treatment failure is generally associated with increased MICs (10); however, conventional susceptibility testing based on a single critical concentration is unable to distinguish between borderline and high-level resistance (21). Farhat et al. showed that up to 50% of *M. tuberculosis* strains had discrepant drug susceptibility testing results when tested for susceptibility to ciprofloxacin, OFX, and MFX (24). Based on those findings, the authors suggested that fluoroquinolone-specific MICs should be measured to estimate fluoroquinolone resistance (24). Although moderate increments in MICs do not always correlate with therapeutic failure, small increases in MIC values can indicate a gradual accumulation of mutations in critical genes, resulting in highly resistant strains (9). Therefore, routine measurement of the MICs of MFX and companion drugs is necessary to avoid exposing patients with infections due to *M. tuberculosis* to subinhibitory MFX concentrations (25).

This study has several limitations. First, the anti-TB regimens evaluated in this study were heterogeneous because of the retrospective nature of the study. Second, this was a nonrandomized

study with a relatively small number of patients. Because of the small number of cases, the lack of benefit of using MFX among MDR-TB patients whose isolates showed high-level MFX resistance could have been a type II error due to low statistical power. Prospective studies with a larger sample size are needed to determine the efficacy of MFX for the treatment of OFX-resistant MDR-TB.

In conclusion, we found that most (69.1%) MDR-TB isolates determined to be OFX resistant by conventional susceptibility testing were susceptible to or exhibited low-level resistance to MFX and that most patients who received MFX benefited from said treatment. Our results indicate that MFX-based regimens are effective against OFX-resistant MDR-TB. Our study results also support the new WHO recommendations for testing of MFX susceptibility at two critical concentrations.

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