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Prevalence of Fluoroquinolone Resistance among Tuberculosis Patients in Shanghai, China▽

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We determined the prevalence of fluoroquinolone resistance among the isolates of *Mycobacterium tuberculosis* from 605 pulmonary tuberculosis patients in Shanghai, China. Mutations in *gyrA* were found in 81.5% of phenotypically fluoroquinolone-resistant isolates and were used as a molecular marker of fluoroquinolone resistance. *gyrA* mutations were detected in 1.9% of strains pan-susceptible to first-line drugs and 25.1% of multidrug-resistant strains. Fluoroquinolone resistance was independently associated with resistance to at least one first-line drug and prior tuberculosis treatment.

Fluoroquinolones are among the most promising antibiotic drugs for tuberculosis (TB) treatment and have the potential to become part of a new first-line treatment regimen against TB (12, 17). Fluoroquinolones were introduced into clinical practice in China nearly 20 years ago and have been widely used to treat common bacterial infections, TB patients infected with *Mycobacterium tuberculosis* strains resistant to first-line drugs, and TB patients with severe adverse reaction to first-line agents (2, 13). Although high levels of fluoroquinolone resistance have been detected among many common bacterial pathogens (16, 19), little is known about the fluoroquinolone resistance of *M. tuberculosis*. A previous study reported that the risk that a TB patient would acquire fluoroquinolone resistance was correlated with the patient's previous exposure to fluoroquinolones (14). If TB patients are infected with *M. tuberculosis* strains that are resistant to fluoroquinolones, it will not be possible to use fluoroquinolones in anti-TB treatment regimens.

To estimate the prevalence and to identify the risk factors associated with fluoroquinolone resistance among pulmonary TB patients in Shanghai, we performed a retrospective case control study using specimens and data collected and stored in the Tuberculosis Reference Laboratory, Shanghai Municipal Center for Disease Control and Prevention. The incidence rate of pulmonary TB in Shanghai in 2005 was 39.4 per 100,000 persons. From March 2004 through November 2007, clinical isolates from 4,663 patients with pulmonary TB were collected. Drug susceptibility testing for the major first-line drugs, specifically, isoniazid (0.2 μg/ml), rifampin (rifampicin) (40 μg/ml), ethambutol (2 μg/ml), and streptomycin (4 μg/ml), were routinely performed on each isolate by using the proportion method (4). A total of 85.1% of the TB patients were infected with *M. tuberculosis* strains susceptible to isoniazid, rifampin, ethambutol, and streptomycin; these patients are hereafter referred to as pan-susceptible. A total of 14.9% of the strains were resistant to at least one first-line drug, and 5.6% were multidrug resistant (MDR) (18). We selected a random sample of TB patients infected with pan-susceptible strains ($n = 257$), a random sample of TB patients infected with a strain monoresistant to isoniazid ($n = 60$), and a random sample of TB patients infected with a strain that was polyresistant but not MDR ($n = 77$), all TB patients infected with a strain monoresistant to rifampin ($n = 36$), and all 175 (70.9%) strains available from 247 reported MDR-TB patients. In total, the initial clinical isolates of *M. tuberculosis* and sociodemographic data from 605 pulmonary TB patients were included in the study. The study was approved by the ethics committee of Fudan University.

Mutations in the fluoroquinolone resistance-determining region of *gyrA* gene are the most important mechanism of fluoroquinolone resistance in *M. tuberculosis* (1, 7, 8, 11). To confirm that mutations in the fluoroquinolone resistance-determining region of *gyrA* can be used as a reliable molecular marker for detection of fluoroquinolone-resistant *M. tuberculosis* in Shanghai, we compared ofloxacin (2 μg/ml) susceptibility testing and *gyrA* sequencing results for 175 MDR strains. The sensitivity of *gyrA* mutations among 54 isolates with the ofloxacin-resistant phenotype was 81.5% (95% confidence interval [CI], 68.6% to 90.8%). The specificity of *gyrA* sequencing was 100% (97.5% CI, 97.0% to 100.0%). Next, we sequenced the *gyrA* gene of the initial isolate from 605 pulmonary TB patients (Table 1). The prevalence of *gyrA* mutations was low-

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TABLE 1. Estimates of prevalence levels and 95% CIs for *gyrA* mutations by drug susceptibility test result

Characteristic[a]	No. of isolates in study population		% Prevalence estimate (95% CI)
	Total	With *gyrA*	
Pan-susceptibility	257	5	1.9 (0.6–4.5)
Resistance to one or more first-line drugs	348	59	17.0 (13.2–21.3)
Any resistance to INH	302	53	17.5 (13.4–22.3)
Any resistance to RIF	220	50	22.7 (17.4–28.8)
Any resistance to EMB	69	19	27.5 (17.5–39.6)
Any resistance to SM	196	40	20.4 (15.0–26.7)
Monoresistance to INH	60	4	6.7 (1.8–16.2)
Monoresistance to RIF	36	4	11.1 (3.1–26.1)
Polyresistance	77	7	9.1 (3.7–17.8)
Any drug resistance except MDR	173	15	8.7 (4.9–13.9)
MDR	175	44	25.1 (18.9–32.2)

[a] INH, isoniazid; RIF, rifampin; EMB, ethambutol; SM, streptomycin; polyresistance, resistance to two or more first-line drugs but not MDR; MDR, resistance to at least INH and RIF.

TABLE 3. Results of multivariate logistic regression analysis for identification of covariates that were independently associated with *gyrA* mutations, a marker of fluoroquinolone resistance[a]

Characteristic	Adjusted OR (95% CI)	*P*
MDR	13.8 (5.2–36.5)	<0.0005
Monoresistance to rifampin	6.3 (1.6–25.1)	0.010
Polyresistance	4.5 (1.3–14.8)	0.015
Age ≥46 yr	2.4 (1.3–4.4)	0.005
TB retreatment	2.1 (1.2–3.8)	0.014

[a] OR, odds ratio; MDR, resistance to at least isoniazid and rifampin; polyresistance, resistance to two or more first-line drugs but not MDR.

est among pan-susceptible strains of *M. tuberculosis* (1.9%) and higher among strains that were resistant to one or more first-line drugs (17.0%), particularly MDR strains (25.1%). By univariate analysis, *gyrA* mutations were more likely to occur among strains resistant to first-line anti-TB drugs, especially MDR strains, than among pan-susceptible strains (Table 2). By multivariate logistic regression modeling, with adjustment for age, MDR was the strongest independent predictor of a *gyrA* mutation (Table 3). We tested the multivariate model for goodness of fit ($P = 0.607$), and interaction terms did not significantly improve the model.

A history of prior TB treatment was also an independent predictor of *gyrA* mutations (Table 3). Medical records were available for 15 of 25 Shanghai residents but none of the 7 migrants with retreatment TB and a *gyrA* mutation. For 53% (8/15) of the TB patients whose medical records were re-

TABLE 2. Results of univariate analysis of characteristics of TB patients by presence or absence of *gyrA* mutation, a marker of fluoroquinolone resistance

Characteristic[a]	No. (%) of isolates with *gyrA* mutation		OR[c] (95% CI)	*P*
	Present ($n = 64$)	Absent ($n = 541$)		
Drug susceptibility				
Pan-susceptibility	5 (7.8)	252 (46.6)	1.0	
Resistance to one or more drugs[b]	59 (92.2)	289 (53.4)	10.3 (4.1–33.3)	<0.00005
Any INH resistance[b]	53	249	10.7 (4.2–34.9)	<0.00005
Any RIF resistance[b]	50	170	14.8 (5.8–48.4)	<0.00005
Any EMB resistance[b]	19	50	19.2 (6.4–67.8)	<0.00005
Any SM resistance[b]	40	156	12.9 (4.9–42.6)	<0.00005
Monoresistance to INH[b]	4	56	3.6 (0.7–17.2)	0.069
Monoresistance to RIF	4	32	6.3 (1.2–30.6)	0.016
Polyresistance[b]	7	70	5.0 (1.3–20.7)	0.0018
MDR[b]	44	131	16.9 (6.5–55.7)	<0.00005
Any category of drug resistance except MDR[b]	15	158	4.8 (1.6–17.1)	0.0002
MDR vs any other category of drug resistance	44	131	3.5 (1.8–7.1)	0.0000
Patient status				
Retreatment case	32 (50.0)	113 (20.9)	3.8 (2.1–6.7)	<0.00005
New case	32 (50.0)	428 (79.1)	1.0	
Patient origin				
Resident of Shanghai	46 (71.9)	333 (61.6)	1.6 (0.9–3.0)	0.107
Migrant	18 (28.1)	208 (38.4)	1.0	
Age				
≥46 yr	43 (67.2)	264 (48.0)	2.2 (1.2–3.9)	0.005
<46 yr	21 (32.8)	277 (51.2)	1.0	
Sex				
Male	47 (73.4)	409 (75.6)	1.1 (0.6–2.1)	0.704
Female	17 (26.6)	132 (24.4)	1.0	

[a] INH, isoniazid; RIF, rifampin; EMB, ethambutol; SM, streptomycin; polyresistance, resistance to two or more first-line drugs but not MDR; MDR, resistance to at least INH and RIF.
[b] Pan-susceptible isolates were used as the reference group.
[c] OR, odds ratio.

viewed, there was documentation indicating that the patient had received at least 2 weeks of fluoroquinolone treatment in a previous TB treatment regimen. Fluoroquinolone use during anti-TB treatment likely contributed to acquired fluoroquinolone resistance in retreatment patients.

The classical fluoroquinolone drug susceptibility test recommended by the World Health Organization is the proportion method (5, 15), but this method is relatively time-consuming and labor-intensive. The positive predictive value of screening for *gyrA* mutations to detect fluoroquinolone resistance varies, depending on the study site and sampling, the prevalence of fluoroquinolone resistance, and the study design. Eighty-six percent (23/30) of fluoroquinolone-resistant isolates in a study performed in North America had a *gyrA* mutation (3), but only 35.7% (5/14) fluoroquinolone-resistant isolates had *gyrA* mutation in a study with a small sample of fluoroquinolone-resistant patients in Taiwan (14). A study in Beijing, China, that used denaturing high-pressure liquid chromatography and DNA sequencing reported that 56% of 87 ofloxacin-resistant *M. tuberculosis* clinical strains had a mutation in *gyrA* (10). In our study, the sensitivity of the *gyrA* mutation for detection of the ofloxacin-resistant phenotype was 81.5%, and this molecular marker was reasonable for prediction of phenotypic fluoroquinolone resistance. However, we did not screen for all possible mutations that have been reported to confer fluoroquinolone resistance, such as mutations in the *gyrB* gene and the efflux pump (6, 9, 14), and the mechanisms of fluoroquinolone resistance in *M. tuberculosis* are still not fully understood. *gyrA* mutations do not perfectly predict fluoroquinolone resistance phenotypes, and we may have underestimated the true prevalence of fluoroquinolone resistance in our study population.

Fluoroquinolones have the potential to become part of a new first-line treatment regimen against TB but will not be effective if the prevalence of fluoroquinolone resistance among new TB cases is high. In our retrospective study, 1.9% of the pan-susceptible strains of *M. tuberculosis* from pulmonary TB patients in Shanghai had a *gyrA* mutation. Fluoroquinolone resistance was independently associated with resistance to first-line drugs and prior TB treatment. Although our data did not permit a statistical comparison, fluoroquinolone resistance is likely to be associated with prior fluoroquinolone use during prior TB treatment. Inappropriate regimens, such as monotherapy or a regimen delivered without directly observed therapy, likely contribute to acquired fluoroquinolone resistance in *M. tuberculosis*. Thus, more attention should be paid to fluoroquinolone use and potential acquired fluoroquinolone resistance during anti-TB therapy, especially in populations where TB treatment guidelines are not well established and MDR TB occurs.

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