

Additional benefits of GeneXpert MTB/RIF assay for the detection of pulmonary tuberculosis patients with prior exposure to fluoroquinolones

Peijun Tang^{1,*}

Ping Xu^{1,*}

Wei Shu²

Xiafang Wang¹

Jian Guo¹

Huafeng Song¹

Sumei Li¹

Yu Pang²

Meiying Wu¹

¹Department of Tuberculosis, The Fifth People's Hospital of Suzhou, Suzhou, China; ²National Clinical Laboratory on Tuberculosis, Beijing Chest Hospital, Capital Medical University, Beijing Tuberculosis and Thoracic Tumor Institute, Beijing, China

*These authors contributed equally to this work

Background: We performed a prospective study to investigate the association between pre-diagnosis exposure to fluoroquinolone (FQ) and laboratory testing results among tuberculosis (TB) patients.

Patients and methods: Each TB-suspected patient provided sputum specimen for mycobacteria growth indicator tube (MGIT) culture and GeneXpert among pulmonary TB patients. Confirmed TB patients and clinically diagnosed TB patients were further enrolled in the final analysis.

Results : A total of 661 TB patients were included in the final analysis. The distribution of rural TB patients in the FQ-exposed group was significantly higher than that of urban TB patients ($P=0.02$). GeneXpert showed significantly better positive rate than MGIT technology for TB cases with prior FQ exposure (30.6% for GeneXpert vs 20.1% for MGIT, $P=0.01$). The positive rate of GeneXpert was significantly higher than that of MGIT for 7–13 days ($P=0.04$) and ≥ 14 days FQ exposure ($P=0.01$) groups, respectively. We also found that the positive rate of MGIT was significantly decreased from 31.5% for <7 days levofloxacin (LFX) exposure group to 9.4% for ≥ 14 days LFX exposure group ($P=0.01$), whereas the positive rate of MGIT displayed significant decrease after 7–13 days exposure to moxifloxacin ($P=0.04$).

Conclusion: In conclusion, our data demonstrate that TB patients prior to sputum collection are prone to yield negative culture results. GeneXpert could bring additional benefits for the detection of pulmonary TB patients with prior exposure to FQs. In addition, the exposure to moxifloxacin affected mycobacterial culture at an earlier stage compared with LFX.

Keywords: tuberculosis, fluoroquinolone exposure, GeneXpert

Introduction

Tuberculosis (TB) remains the leading cause of death from a curable infectious disease.^{1,2} It is estimated that a total of 10.4 million new TB cases occurred and ~1.7 million cases died of this disease worldwide in 2016.¹ China has the world's third largest TB epidemic, accounting for 11% of global TB incidence.¹ Although China has experienced dramatic decline in TB prevalence during the past 20 years,³ the emergence of drug-resistant TB hampers efforts at TB control in this country.⁴ The inappropriate use of antibiotics is a major driving force behind the epidemic of drug-resistant TB, especially for fluoroquinolones (FQs).^{5,6}

FQs demonstrate excellent in vitro and in vivo activity against *Mycobacterium tuberculosis* (MTB) and have become cornerstone drugs for the treatment of drug-resistant TB.^{5,7} Unlike other anti-TB agents, FQs are also one of the most widely used antibiotics for a broad spectrum of bacterial infections, especially for treating undiagnosed respiratory

Correspondence: Yu Pang
Beijing Chest Hospital, Capital Medical University, Beijing Tuberculosis and Thoracic Tumor Institute, No. 97, Machang, Tongzhou District, Beijing, 101149, China
Tel +86 10 8950 9359
Fax +86 10 8950 9359
Email pangyupound@163.com

Meiying Wu
The Fifth People's Hospital of Suzhou, No. 10, Changqian Road, Xiangcheng District, Suzhou, 215100, China
Tel +86 512 6518 0352
Fax +86 512 6518 0352
Email wu_my@126.com

bacterial infections.^{8,9} As a consequence, the empirical therapy of suspected infection possibly with FQs masks the diagnosis of active TB, thereby leading to delay in initiation of anti-TB treatment and enhanced TB transmission within the community.⁹

Administration of FQs would transiently reduce the bacterial load and impair the vitality of mycobacteria in patients.^{7,9} Decreased vitality of mycobacteria in the specimen will undoubtedly result in the loss of recovery rate by mycobacterial culture. In contrast, nucleic acid amplification tests have no requirement for viability of mycobacteria; prior exposure to FQ would thus cause less impact on the detection of MTB by molecular diagnostics in comparison with conventional culture. In order to test the null hypothesis, we performed a prospective study to investigate the association between pre-diagnosis exposure to FQ and laboratory testing results by mycobacteria growth indicator tube (MGIT) culture and GeneXpert among pulmonary TB patients.

Patients and methods

Patients

We performed a prospective observational study among TB suspected patients in the Fifth People's Hospital of Suzhou, China, from January 2017 to December 2017. A TB-suspected patient was defined as someone presenting at the clinic with a cough of 2 weeks or more.¹⁰ Confirmed TB patients and clinically diagnosed TB patients were further enrolled in final analysis. The definition of TB patients followed the guidelines endorsed by WHO.¹¹ We interviewed the patients to collect a comprehensive listing of FQ prescriptions before TB diagnosis. In this setting, third-generation FQs, including levofloxacin (LFX; 500 mg/day) and moxifloxacin (MOX; 400 mg/day), are used for the empirical therapy of suspected respiratory infections. For each TB patient who received FQ exposure, we identified nonexposed patients as the control group. We collected data on age, sex, residence, previous history of TB and exposure history to FQ.

Laboratory examination

Each patient provided sputum specimen for laboratory examination. Direct smears of sputum specimen were examined using Auramine O staining for acid-fast bacilli.¹² Sputum specimens were then processed with *N*-acetylcysteine-citrate-NaOH for 15 minutes and were neutralized with PBS (0.067 mol/L, pH =7.4), with subsequent centrifugation at 4,000× *g* for 15 minutes. The supernatants were discarded, and the sediments were resuspended in 2 mL PBS. Then, 0.5 mL of suspended sputum sediment was used to inoculate one MGIT tube.¹⁰

Bacterial colonies were harvested 4–8 weeks later for conventional drug susceptibility testing by proportional method as previously reported.¹³ The concentrations of anti-TB drugs tested in this study were as follows: isoniazid, 0.2 µg/mL; rifampin (RIF), 40 µg/mL; ethambutol, 2 µg/mL; streptomycin, 4 µg/mL; amikacin, 30 µg/mL; and LFX, 2 µg/mL. The strain was declared resistant to the specific drug if the growth rate was >1% compared to the control.¹⁴

GeneXpert testing (Cepheid, Sunnyvale, CA, USA) was performed following the manufacturer's instructions. Briefly, sample reagent was added to sputum specimen at a ratio of 2:1. The decontaminated sample was vortexed vigorously twice during 15 minutes of incubation at room temperature. Then, 2 mL of the mixture was transferred to the test cartridge and loaded into GeneXpert platform. The results were automatically interpreted by the GeneXpert system after 90 minutes.

Statistical analysis

Statistical analyses were performed using the SPSS software package, version 20.0 (IBM Corporation, Armonk, NY, USA). Chi-squared test or Fisher's exact test was used to compare categorical variables. A *P*-value <0.05 was considered statistically significant.

Ethics statement

This study was approved by the Ethics Committee of the Fifth People's Hospital of Suzhou, and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from each patient enrolled in this study.

Results

Patients

In total, 951 patients with suspected TB were consecutively enrolled in this analysis. By reviewing the laboratory examinations, demographic and clinical data, 290 patients were excluded due to non-TB (*n*=174), lack of exposure data to FQ (*n*=86), contaminated culture results (*n*=21) or invalid GeneXpert results (*n*=9). Of 661 TB patients included in the final analysis, 432 patients (65.4%) experienced no FQ exposure during the course of disease, whereas the other 229 (34.6%) had prior exposure to FQ, 163 (71.2%) and 66 (28.8%) of whom were exposed to LFX and MOX, respectively (Figure 1).

Factors associated with FQ exposure

We first compared the distribution of demographic characteristics and in vitro susceptibility between FQ exposed and nonexposed groups. As shown in Table 1, the TB patients

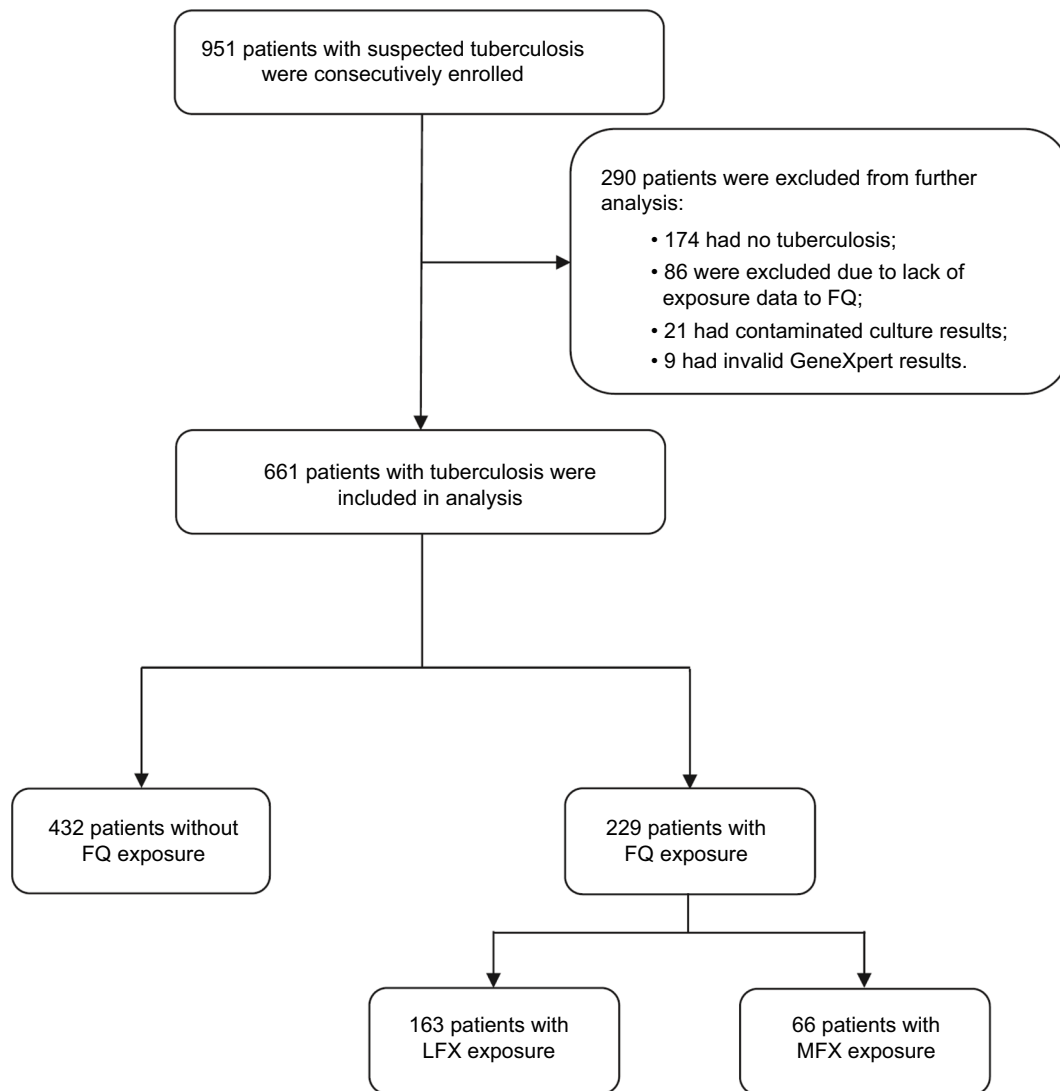


Figure 1 Enrollment of patients in this study.

Abbreviations: FQ, fluoroquinolone; LFX, levofloxacin; MOX, moxifloxacin.

aged 45–64 years (OR [95% CI]: 1.80 [1.21–2.67], $P=0.01$) and ≥ 65 years (OR [95% CI]: 1.73 [1.07–2.80], $P=0.03$) were more likely to be exposed to FQ compared with those aged 25–44 years. In addition, we observed that the distribution of rural TB patients in the FQ-exposed group was significantly higher than that of urban TB patients (OR [95% CI]: 1.49 [1.08–2.06], $P=0.02$). In contrast, no statistical difference was found in the distribution of FQ-exposed patients in gender and drug susceptibility category ($P>0.05$).

Effect of FQ exposure on detection of MTB by liquid culture and GeneXpert

One hundred and fifty-four (35.6%) of 432 TB patients without FQ exposure were detected by the MGIT 960 test, which was similar to that (34.3%, 148/432) by the GeneXpert test

($P>0.05$). For TB cases with prior FQ exposure, the positive rates of MGIT and GeneXpert for detection of MTB were 20.1% (46/229) and 30.6% (70/229), respectively. The statistical analysis revealed that GeneXpert showed significantly better positive rate than MGIT technology for these cases ($P=0.01$).

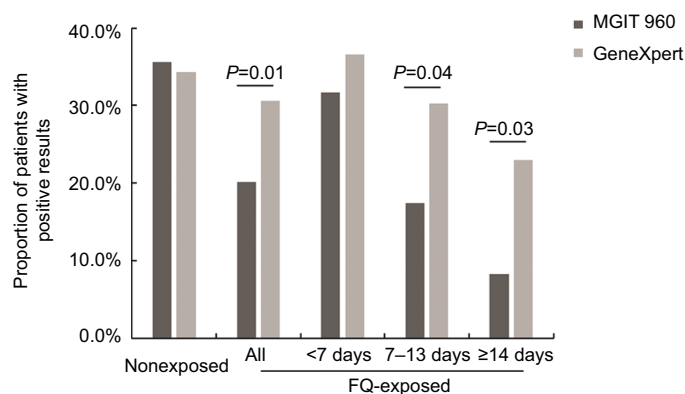
We further analyzed the positive rate according to different FQ exposure duration. As shown in Figure 2, the duration of FQ exposure had no significant effect on the positive rate between MGIT (31.7%, 26/82) and GeneXpert (36.6%, 30/82) for the patients with <7 days of FQ exposure ($P>0.05$). On the contrary, the positive rate of GeneXpert was significantly higher than that of MGIT for 7–13 days (17.4% for MGIT vs 30.2% for GeneXpert, $P=0.04$) and ≥ 14 days (8.2% for MGIT vs 23.0% for GeneXpert, $P=0.03$) groups, respectively.

Table 1 Demographic and clinical characteristics of TB patients enrolled in this study

Characteristics	No. of patients (%)			ORs (95% CI)	P-value
	FQ-exposed group (n=229)	FQ-nonexposed group (n=432)	Total n (%)		
Age (years)					
<25	32 (14.0)	87 (20.1)	119 (18.0)	0.87 (0.54–1.41)	0.57
25–44	80 (34.9)	189 (43.8)	269 (40.7)	Ref.	–
45–64	76 (33.2)	100 (23.1)	176 (26.6)	1.80 (1.21–2.67)	0.01
≥65	41 (17.9)	56 (13.0)	97 (14.7)	1.73 (1.07–2.80)	0.03
Gender					
Male	160 (69.6)	274 (63.4)	434 (65.7)	1.34 (0.95–1.89)	0.10
Female	69 (30.1)	158 (36.6)	227 (34.1)	Ref.	–
Residence					
Rural	94 (41.0)	220 (50.9)	314 (47.5)	Ref.	–
Urban	135 (59.0)	212 (49.1)	347 (52.5)	1.49 (1.08–2.06)	0.02
Resistance to: ^a					
RIF	3 (6.5)	11 (7.1)	14 (7.0)	0.91 (0.24–3.40)	1.00
INH	5 (10.9)	18 (11.7)	23 (11.5)	0.92 (0.32–2.64)	0.88
SM	7 (15.2)	25 (16.2)	32 (16.0)	0.93 (0.37–2.30)	0.87
EMB	2 (4.3)	5 (3.2)	7 (3.5)	1.36 (0.25–7.22)	0.66
LFX	2 (4.3)	7 (4.5)	11 (4.5)	0.96 (0.19–4.76)	1.00
AMK	2 (4.3)	6 (3.9)	8 (4.0)	1.12 (0.22–5.75)	1.00

Note: ^aWe calculated the proportion of resistant organisms based on the MTB isolates with in vitro susceptibility testing results.

Abbreviations: AMK, amikacin; EMB, ethambutol; FQ, fluoroquinolone; INH, isoniazid; LFX, levofloxacin; RIF, rifampin; SM, streptomycin; TB, tuberculosis.

**Figure 2** Comparison of detection rates for MTB between GeneXpert and MGIT according to FQ exposure stratification.

Abbreviations: FQ, fluoroquinolone; MTB, *Mycobacterium tuberculosis*; MGIT, mycobacteria growth indicator tube.

Comparison of LFX and MOX exposure on the detection of MTB

Further analysis was performed to determine whether LFX and MOX exposure produced different impact on the detection of MTB. We found that the positive rate of MGIT was decreased from 31.5% (17/54) for <7 days LFX exposure group to 9.4% (5/53) for ≥14 days LFX exposure group, and the difference was statistically significant between <7 and ≥14 days groups ($P=0.01$). For patients with prior exposure to MOX, the statistical analysis revealed that the positive rate of MGIT displayed significant decrease after 7–13 days of

exposure to MOX (10.0%, 3/30) compared with that in <7 days exposure group (32.1%, 9/28; $P=0.04$). In contrast, no significant difference was observed in the detection rate of GeneXpert for MTB between these groups ($P>0.05$; Table 2).

Discussion

The escalating use of FQs for routine bacterial infections is associated with possible masking of active TB.^{9,15} We examined the impact of FQ use on the detection rates of GeneXpert and MGIT for MTB. Our data demonstrated that TB patients who received FQ for ≥7 days prior to sputum collection were

Table 2 Laboratory results by MGIT 960 and GeneXpert among patients with exposure to FQ

Group	Laboratory method	No. of patients with positive laboratory results (%) ^a		
		<7 days	7–13 days	≥14 days
LFX	MGIT 960	17 (31.5)	13 (23.2)	5 (9.4)*
	GeneXpert	20 (37.0)	17 (30.4)	13 (24.5)
	Total	54 (100.0)	56 (100.0)	53 (100.0)
MOX	MGIT 960	9 (32.1)	3 (10.0)*	1 (12.5)
	GeneXpert	10 (35.7)	9 (30.0)	1 (12.5)
	Total	24 (100.0)	30 (100.0)	8 (100.0)

Note: ^aThe asterisks represent statistically significant differences from the control (patients without exposure to FQ).

Abbreviations: FQ, fluoroquinolone; LFX, levofloxacin; MOX, moxifloxacin; MGIT, mycobacteria growth indicator tube.

more prone to yield negative culture results than nonexposed patients. Similar to our observations, a recent study from South Africa revealed that patients recently exposed to ≥ 5 days of FQ were less likely to be smear positive, resulting in an increased time to treatment.⁹ In addition, GeneXpert exhibited better sensitivity to detect MTB from those with ≥ 7 days exposure to FQ, suggesting that GeneXpert could bring additional benefits for the diagnosis of TB patients with prior exposure to FQ. This appeared to be mainly because the molecular diagnostics does not depend on the presence of viable organisms. There is no doubt that the tubercle bacillus cells undergo loss of viability when exposed to FQs, and the prolonged duration of initial empirical FQ treatment is associated with lower recovery rate in clinical specimens. However, several previous reports demonstrated that $>50\%$ of the bacilli observed on smear microscopy after the 2-month intensive phase were not alive,^{16,17} indicating that the dead tubercle bacillus persists in the pulmonary lesion despite appropriate treatment and apparent recovery. As a consequence, GeneXpert MTB/RIF assay could be used as an alternative for the detection of MTB for patients with prior exposure to FQs.

Another interesting finding of this study is that the exposure to MOX affected mycobacterial culture at an earlier stage compared with LFX, reflecting that MOX has superior overall efficacy over LFX against MTB. This hypothesis is confirmed by other in vitro and in vivo experimental results.^{18–20} On one hand, two animal studies have suggested that MOX concentrates inside the macrophages and pulmonary lesions to a greater extent than LFX.^{19,21} Hence, the greater potency of MOX relative to LFX majorly contributed to the improved drug delivery to the sites of infection. On the other hand, FQs exhibit dose- and concentration-dependent killing kinetics against MTB and other pathogens.^{22–24} A recent study showed that LFX at 1,000 mg daily yielded a similar early bactericidal

activity to a 400 mg daily dose of MOX.¹⁹ Hence, the reduced 500 mg daily dose of LFX may be less potent than 400 mg daily dose of MOX against MTB in clinical practice, which may be an additional reason for the greater EBA of MOX in our observations.

Previous studies have demonstrated potential detrimental effects of FQ use in patients with undiagnosed TB, including an increased risk of diagnosis delay and mortality.^{7,9} In this study, rural residence was associated with greater FQ prescription rate prior to TB diagnosis, which was also an important determinant of patient delay in seeking and receiving care for TB in China.²⁵ Despite the implementation of New Cooperative Medical System in rural China since 2003, rural patients have to suffer high out-of-pocket costs on diagnosis and treatment for TB.^{26,27} Therefore, the poor economic status becomes a major barrier to access to health care for patients living in rural areas. In addition, the significant quality deficits among village clinics and township health centers in the management of TB patients have been reported by Sylvia et al,²⁸ and the sizable gap between physician knowledge and practice would lead to the incorrect interpretations of clinical symptoms associated with TB, thereby resulting in the empirical treatment with antibiotics. Given that rural areas have consistently higher TB prevalence than urban areas in China, concerns have been raised regarding how to take effective actions to protect the most vulnerable members of the population, particularly the rural poor.

This study has several obvious limitations. First, this study was only carried out in a single hospital. The small sample size of patients with MOX exposure may undermine the reliability of our observation. Second, the patients enrolled in this study had a fixed-dose LFX or MOX. It is difficult to conclude whether increased dose of FQ would accelerate the reduction of viable MTB in pulmonary lesions, thereby affecting the performance of laboratory examination. Third, we also found that $\sim 10\%$ of TB patients with long-period FQ exposure (≥ 14 days) yielded positive culture results. Unfortunately, the molecular characteristics of *gyrA* and *gyrB* conferring FQ resistance and minimal inhibitory concentrations were not analyzed in our study.^{5,29} Fourth, although GeneXpert could identify additional TB cases with prior exposure to FQ, some TB cases failed to be detected by both diagnostic tests. The major explanation may be due to the fact that the GeneXpert assay targets a single copy gene of *rpoB*, which may be associated with the decreased sensitivity compared to the multicopy MTB target.³⁰ Recently, a new-generation GeneXpert Ultra has been developed by adding additional multicopy amplification targets (IS6110 and IS1081).³¹ The

application of this novel test will serve as an alternative way that the additional cases could be identified. Fifth, it should be noted that GeneXpert cannot differentiate between live and dead tubercle bacilli, thereby resulting in increasing probability of false-positive cases. Despite these limitations, a number of important clinical implications flow from our findings. On one hand, we present a new laboratory diagnostic diagram for TB suspects with FQ exposure. On the other hand, the high rate of FQ exposure prior to TB diagnosis highlights the urgent need to strengthen the guidance of FQ prescription used for empirical treatment against bacterial infections.

In conclusion, our data demonstrate that TB patients who received FQ for ≥ 7 days prior to sputum collection are more prone to yield negative culture results than nonexposed patients. As an alternative, GeneXpert MTB/RIF assay could bring additional benefits for the detection of pulmonary TB patients with prior exposure to FQs. In addition, the exposure to MOX affected mycobacterial culture at an earlier stage compared with LFX. The high rate of FQ exposure prior to TB diagnosis highlights the urgent need to strengthen the guidance of FQ prescription used for empirical treatment against bacterial infections.

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Disclosure

The authors report no conflicts of interest in this work.

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