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Fluoroquinolone susceptibility in *Mycobacterium tuberculosis* after pre-diagnosis exposure to older- versus newer-generation fluoroquinolones☆

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Abstract

Fluoroquinolone exposure before tuberculosis (TB) diagnosis is common. We anticipated that exposure to older-generation fluoroquinolones is associated with greater fluoroquinolone MICs in *Mycobacterium tuberculosis* than exposure to newer agents. A nested case–control study was performed among newly diagnosed TB patients reported to the Tennessee Department of Health (January 2002–December 2009). Each fluoroquinolone-resistant case ($n = 25$) was matched to two fluoroquinolone-susceptible controls ($n = 50$). Ciprofloxacin and ofloxacin were classified as older-generation fluoroquinolones; levofloxacin, moxifloxacin and gatifloxacin were considered newer agents. There was no difference between median ofloxacin MIC for isolates from 9 patients exposed only to older fluoroquinolones, 25 exposed only to newer fluoroquinolones, 6 exposed to both and 35 fluoroquinolone-unexposed patients (Kruskal–Wallis, $P = 0.35$). Using multivariate proportional odds logistic regression adjusting for age and sex, duration of exposure to newer fluoroquinolones was independently associated with higher MIC (OR = 1.79, 95% CI 1.22–2.64), but duration of exposure to older fluoroquinolones was not (OR = 0.94, 95% CI 0.50–1.78).

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Isolates from patients exposed only to newer fluoroquinolones tended to have mutations at *gyrA* codons 90, 91 or 94 more frequently than those exposed only to older fluoroquinolones (44% vs. 11%). We were surprised to find that duration of exposure to newer fluoroquinolones, but not older ones, was independently associated with higher ofloxacin MIC. This suggests that the mutant selection window lower boundary is likely to have clinical relevance; caution is warranted when newer fluoroquinolones are prescribed to patients with TB risk factors.

Keywords

Drug-resistant tuberculosis; Genotypic resistance; Moxifloxacin; Levofloxacin; Ciprofloxacin

1. Introduction

Fluoroquinolones are a critical component of antituberculous drug regimens for multidrug-resistant (MDR) (defined as *Mycobacterium tuberculosis* isolates with resistance to isoniazid and rifampicin) tuberculosis (TB) and are being considered for use as first-line anti-TB therapy for drug-susceptible disease [1,2]. Fluoroquinolones have broad antimicrobial activity and are widely used to treat a variety of bacterial infections [3]. Exposure to fluoroquinolones prior to the diagnosis of TB has been associated with fluoroquinolone resistance, particularly when fluoroquinolone exposure occurs >60 days before TB diagnosis and for longer than 10 days [4,5]. However, it is unknown whether the specific fluoroquinolone prescribed before TB diagnosis makes a difference in the subsequent development of fluoroquinolone resistance.

Chemical modifications of nalidixic acid have led to the development of three structural generations of fluoroquinolones, although a number of other approaches to quinolone classification have been used [6,7]. One such classification, which we have used for the current study, distinguishes newer from older fluoroquinolones based on general clinical pharmacological differences; newer fluoroquinolones, such as levofloxacin, moxifloxacin and gatifloxacin, have longer serum half-lives, achieve higher peak levels and have higher volumes of distribution than older fluoroquinolones, such as ofloxacin and ciprofloxacin [8]. Although all of the fluoroquinolones have demonstrated in vitro activity against *M. tuberculosis*, older fluoroquinolones, especially ciprofloxacin, have lower sterilising and early bactericidal activity against *M. tuberculosis* than newer fluoroquinolones [9]. In addition, newer agents have lower minimum inhibitory concentrations (MICs) than older fluoroquinolones [10].

Spontaneous mutations in *M. tuberculosis* isolates that confer fluoroquinolone resistance are often present at low levels in untreated patients with TB, allowing fluoroquinolone-resistant subpopulations to be selected and amplified during TB treatment [11]. Individual fluoroquinolones have varying abilities to suppress the amplification of resistant mutant subpopulations. In vitro and modelling studies have shown that ciprofloxacin allows resistant mutant enrichment, whilst high doses of moxifloxacin suppress mutant growth [12,13]. Fluoroquinolone resistance mutations in *M. tuberculosis* most commonly occur in the *gyrA* and *gyrB* genes [14]. The quinolone resistance-determining region (QRDR) of *gyrA* is a conserved region across bacterial species in which fluoroquinolone resistance mutations frequently occur, the most common of which are at codons 90, 91 and 94 in *M. tuberculosis* [14]. Specific mutations in the QRDR of *gyrA,* particularly at codon 94, have been associated with higher fluoroquinolone MICs [15,16].

In this study, exposure to older- and newer-generation fluoroquinolones before TB diagnosis and the association with fluoroquinolone MIC against *M. tuberculosis* was assessed. Owing

to the higher MIC, shorter half-life and poor suppression of mutant growth of the older fluoroquinolones, it was expected that exposure to older-generation fluoroquinolones would be associated with higher fluoroquinolone MICs and more frequent *gyrA* mutations (especially at codon 94) than exposure to newer fluoroquinolones. We were surprised to find the opposite, although these findings are readily explained by the mutant selection window hypothesis [17,18].

2. Methods

2.1. Study design

The current study builds upon prior analyses using previously identified *M. tuberculosis* strains [4,19–21]. A nested case–control study that included patients diagnosed with TB and reported to the Tennessee Department of Health between January 2002 and December 2009 was performed. Patients who had fluoroquinolone-resistant *M. tuberculosis* isolates during the study period were considered to be cases. Each case was matched with two patients who had fluoroquinolone-susceptible *M. tuberculosis* isolates from the same year of diagnosis. One of each pair of controls had documented fluoroquinolone exposure in the 12 months prior to TB diagnosis and the other did not. The study was approved by the institutional review boards of Vanderbilt University (Nashville, TN), the Tennessee Department of Health, and the Davidson County Metro Public Health Department (Nashville, TN).

2.2. Fluoroquinolone exposure

Fluoroquinolone exposure was ascertained from one or more of the following sources: the TennCare (Medicaid) pharmacy database; a fluoroquinolone exposure assessment form; an in-home questionnaire; clinic record review; and hospital record review. The TennCare pharmacy database records information for outpatient and emergency department prescriptions. TennCare prescription insurance covered all formulary medications from January 2002 to July 2005, and subsequently covered three generic and two non-generic medications per month. In addition, starting in January 2006, all patients age ≥65 years received prescription benefits through Medicare rather than TennCare. TennCare data were available for 2002–2009; the remaining exposure ascertainment methods were available for individuals diagnosed with TB from 2007–2009. The hospital record review provided inpatient exposure data; the clinic record review provided outpatient exposure data; and the two patient survey tools provided both inpatient and outpatient exposure data.

2.3. Mycobacterium tuberculosis isolate testing

The Tennessee Public Health Laboratory (Nashville, TN) tested all *M. tuberculosis* isolates. Isolates were grown on either Lowenstein–Jensen (LJ) slants or Middlebrook 7H10 or 7H11 plated media. High-performance liquid chromatography or nucleic acid hybridisation (AccuProbe®; Gen-Probe, Inc.) identified mycobacteria depending on the amount of growth present or culture morphology. All clinical *M. tuberculosis* isolates were stored at the Tennessee State Mycobacteriology Laboratory at −70 °C. Isolates were thawed and subcultured onto LJ medium. A 1.0 McFarland inoculum was prepared from colonies on the LJ slant and served as the standard inoculum for susceptibility testing. A critical concentration of 2 μg/mL for ofloxacin was used to categorise susceptible and resistant isolates. The ofloxacin MIC was determined by the indirect agar proportion method using concentrations of 0.5–2 μg/mL for susceptible isolates and 4–1024 μg/mL for resistant isolates.

Genetic sequencing to assess polymorphisms and mutations in *gyrA* and *gyrB* was performed via Sanger sequencing as described previously [19,20]. The QRDR of *gyrA* was

considered to extend from codons 74 to 113 [22] and the QRDR of *gyrB* to extend from codons 500 to 538 [23].

2.4. Statistical analysis

Ofloxacin and ciprofloxacin were considered older-generation fluoroquinolones, whilst gatifloxacin, levofloxacin and moxifloxacin were considered newer-generation fluoroquinolones [8]. Differences between fluoroquinolone-exposed and -unexposed groups and between fluoroquinolone-resistant and -susceptible groups were evaluated using the χ^2 test for categorical variables and the Wilcoxon rank-sum test for continuous variables. Differences in MIC among multiple categories were calculated using the Kruskal–Wallis test. Multivariate proportional odds logistic regression was used to assess the association between MIC and duration of fluoroquinolone exposure with adjustment for age and sex. Statistical analyses were performed using STATA v.10 (StataCorp LP, College Station, TX) and R version 2.10.0 (http://www.r-project.org). All *P*-values were two-sided, and *P*-values of <0.05 were considered significant.

3. Results

Among 2169 cases of TB reported to the Tennessee Department of Health between 2002 and 2009, 1632 (75.2%) were culture-positive. Among all of the culture-positive isolates, 1114 were tested for ofloxacin susceptibility. Only those culture-positive isolates from patients in the TennCare database were tested for ofloxacin susceptibility from 2002–2006. Of the isolates tested for ofloxacin susceptibility, 25 (2.2%) were fluoroquinolone-resistant and designated as case isolates (median MIC 64 μg/mL; MIC range 8–256 μg/mL). The 50 fluoroquinolone-susceptible control isolates had a median MIC of 1 μg/mL (MIC range <0.5 μg/mL to 2 μg/mL). Of these, 24 (48%) had documented fluoroquinolone exposure before TB diagnosis. One control was found after matching to have had fluoroquinolone exposure after, not before, TB diagnosis. Among the 25 fluoroquinolone-resistant cases, 16 (64%) had exposure to any fluoroquinolone before TB diagnosis. Exposure data were available from all five ascertainment methods after 2006; 9 (36%) of the 25 cases occurred after 2006. In a previous publication that included 19 of the 25 fluoroquinolone-resistant isolates in the current study, it was demonstrated that all 19 isolates that were resistant to ofloxacin by the agar proportion method were also resistant to ciprofloxacin, levofloxacin and moxifloxacin using their respective critical concentrations [21].

Patient demographic and clinical information according to fluoroquinolone exposure status is shown in Table 1. Patients who identified themselves as Hispanic or Latino were significantly less likely to be exposed to fluoroquinolones before TB diagnosis; otherwise, there were no statistically significant differences between the groups, including age, sex and human immunodeficiency virus (HIV) status. None of the patients in the study had isolates resistant to isoniazid or rifampicin.

Table 2 characterises the details of fluoroquinolone exposure according to cases and controls. Patients received levofloxacin and ciprofloxacin most frequently, with 25 (33%) and 14 (19%), respectively, of all study patients exposed to these fluoroquinolones before TB diagnosis. Gatifloxacin and ofloxacin were used infrequently. Of nine patients who were exposed only to older fluoroquinolones, eight were exposed to ciprofloxacin and one to ofloxacin. Of 25 patients who were exposed only to newer fluoroquinolones, 19 were exposed to levofloxacin, 8 to moxifloxacin and 2 to gatifloxacin (3 were exposed to both moxifloxacin and levofloxacin, and 1 was exposed to both moxifloxacin and gatifloxacin). Cases (those with fluoroquinolone-resistant *M. tuberculosis*) tended to be more likely to receive a newer-generation fluoroquinolone than controls (those with fluoroquinolone-

susceptible disease) ($P = 0.07$). Among the cases, nine did not have exposure to any fluoroquinolones.

Comparison between cases and controls who received only older fluoroquinolones, only newer fluoroquinolones, both older and newer fluoroquinolones, and no fluoroquinolones showed no difference ($P = 0.3$; Table 2). Similarly, comparison of the median MIC among the four categories of exposure did not differ ($P = 0.35$).

The nine patients exposed only to older fluoroquinolones had a median of 66 days [interquartile range (IQR) 33–83 days] between their last exposure to fluoroquinolones and the diagnosis of TB. For five of the nine patients, this time interval was >60 days. In comparison, the 25 patients exposed only to newer fluoroquinolones had a median of 23 days (IQR 2–70 days) between their last exposure to fluoroquinolones and TB diagnosis. This time interval was >60 days for 7 of the 25 patients.

The median cumulative duration of exposure to fluoroquinolones (summed duration of all courses of fluoroquinolones prescribed per subject) among people exposed exclusively to older fluoroquinolones was 14 days (IQR 7–16 days). Similarly, among those exposed only to newer fluoroquinolones, median exposure was 12 days (IQR 4–22 days). The median MIC of isolates from patients exposed only to older fluoroquinolones was 1 μg/mL (IQR 1–2 μg/mL). The median MIC of isolates from patients exposed only to newer fluoroquinolones was 2 μg/mL (IQR 1–64 μg/mL). The duration of exposure to newer fluoroquinolones was associated with a higher MIC in multivariate proportional odds logistic regression after adjusting for age and sex [odds ratio (OR) = 1.79, 95% confidence interval (CI) 1.22–2.64; $P < 0.01$) (Table 3A). In contrast, the duration of exposure to older fluoroquinolones was not associated with a higher MIC (OR = 0.94, 95% CI 0.50–1.78; $P = 0.86$) (Table 3B).

Among patients with exposure only to older or only to newer fluoroquinolones, all of their associated *M. tuberculosis* isolates had at least one mutation in *gyrA* or *gyrB*. Table 4 shows the differences between isolates from patients who received only older or only newer fluoroquinolones according to the detection of at least one mutation at various locations. Although none of the differences were significant, those with exposure only to newer fluoroquinolones tended to have at least one mutation at codons 90, 91 or 94 more frequently than those with exposure only to older fluoroquinolones [11 (44%) vs. 1 (11%); $P = 0.08$). All of the isolates with mutations at *gyrA* codons 90, 91 or 94 were fluoroquinolone-resistant. None of the fluoroquinolone-resistant isolates had more than one QRDR *gyrA* mutation. Among mutations that occurred at codon 94, Asp94Gly occurred most frequently. Among those exposed to older fluoroquinolones, one isolate had a mutation at codon 94 (Asp94Gly). Among those exposed to newer fluoroquinolones, five had the Asp94Gly mutation, two had Asp94Asn and two had Asp94Tyr. Increasing numbers of mutations in *gyrA* were not associated with higher MIC levels. Among all fluoroquinolone-resistant isolates, only 4 did not have a *gyrA* mutation (median MIC = 128 μg/mL; MIC range 8–256 μg/mL), whilst 21 did have at least one *gyrA* mutation (median MIC = 64 μg/mL; MIC 8–256 μg/mL).

At least one *gyrB* mutation was found in 20 of the fluoroquinolone-resistant isolates and 32 of the fluoroquinolone-susceptible isolates. Only four of the isolates had a mutation in the QRDR of *gyrB*; three of these occurred in susceptible isolates and one of them in a resistant isolate. Only the QRDR *gyrB* mutation that was in the fluoroquinolone-resistant isolate occurred at a codon that has been demonstrated to confer fluoroquinolone resistance, although with a different amino acid substitution (Asn538Ile; previously, Asn538Asp has

been shown to confer fluoroquinolone resistance [24]). This same isolate also had mutations in *gyrA* (Leu625Pro) and *gyrB* (Leu62Pro + Ser421Pro).

The median MIC of the nine fluoroquinolone-resistant isolates from patients who did not have exposure to fluoroquinolones was 128 μg/mL (MIC range 8–256 μg/mL). Six of these nine isolates had a mutation in *gyrA*, three of which had mutations in the QRDR of *gyrA*. Only one isolate had a mutation at codons 90, 91 or 94 (Asp94Asn; also had non-QRDR *gyrA* mutation Asn677Asp and *gyrB* mutation Tyr495Cys; MIC = 256 μg/mL). Six of the nine isolates had a *gyrB* mutation, but none of these were in the QRDR of *gyrB*.

4. Discussion

Given the advantageous pharmacological features of the newer fluoroquinolones, particularly the lower MIC against *M. tuberculosis*, we expected that exposure to these agents before TB diagnosis would be less likely to be associated with a higher MIC than exposure to older-generation fluoroquinolones. We found, however, that although there was no statistically significant difference in MIC associated with exposure to older versus newer fluoroquinolones, the duration of exposure to newer fluoroquinolones was independently associated with greater MIC, whilst duration of exposure to older fluoroquinolones was not. Whilst these findings are surprising, they are plausible when the older and newer fluoroquinolones are considered in the context of the lower boundary of the mutant selection window.

The mutant selection window hypothesis predicts that there is a drug concentration range in which subpopulations of drug-resistant bacterial mutants that are present before antimicrobial administration will be selected and amplified during treatment [17,18]. The lower boundary of the window is the minimum drug concentration that inhibits bacterial growth, approximately equal to the MIC. The upper boundary of the window is known as the mutant prevention concentration, which is the MIC of the least drug-susceptible mutant subpopulation [17]. In vitro work with fluoroquinolones and *Staphylococcus aureus* suggests that resistant mutant subpopulations are selected and enriched when fluoroquinolone concentrations occur inside the mutant selection window [25]. A model using rabbits infected with *S. aureus* supports these findings, demonstrating that levofloxacin-resistant colonies do not emerge as much when levofloxacin concentrations are below the mutant selection window lower boundary as when levofloxacin concentrations are inside the window [26].

With mycobacteria, varying degrees of susceptibility may occur with different resistance mutations at different fluoroquinolone concentrations. In an in vitro study assessing the frequency of *gyrA* and *gyrB* mutations in *M. tuberculosis* and *Mycobacterium smegmatis* isolates exposed to different fluoroquinolone compounds at a range of concentrations, Zhou et al. found that low concentrations of fluoroquinolone (lower portion of the selection window) selected for mostly non-*gyrA* mutants [11]. As fluoroquinolone concentrations increased, however, an increasing number of g*yrA* mutations were identified, with mutations at codon 94 most commonly identified at the highest fluoroquinolone concentrations at which bacilli were still recovered [11]. This work and other studies have demonstrated that mutations at codon 94 in *gyrA* have been associated with decreased susceptibility to fluoroquinolones in comparison with mutations at other *gyrA* codons [15,16,27]. The above findings suggest that exposure to older fluoroquinolones may place *M. tuberculosis* inside the lower portion of the mutant selection window and select for fluoroquinolone resistance mutations that confer lower-level resistance than exposure to newer fluoroquinolones. Therefore, using the MIC as a marker of resistance, exposure to older-generation fluoroquinolones is less likely to result in fluoroquinolone-resistant TB than exposure to

newer-generation agents. In contrast, *M. tuberculosis* isolates from patients exposed to newer fluoroquinolones may fall at the upper region of the mutant selection window and have mutations that confer high-level resistance. In support of this idea, our group previously demonstrated that 13 (93%) of 14 fluoroquinolone-resistant isolates that had at least one mutation at codons 90, 91 or 94 came from patients who had fluoroquinolone exposure prior to TB diagnosis, whilst only 3 (27%) of 11 fluoroquinolone-resistant isolates without such mutations came from patients who had fluoroquinolone exposure ($P = 0.001$) [19,20]. In the current study, we found that isolates from patients exposed only to newer fluoroquinolones tended to have at least one mutation at codons 90, 91 or 94 more frequently than those exposed only to older fluoroquinolones (11/25 (44%) vs. 1/9 (11%); $P = 0.08$]. The low numbers of isolates in the current study likely contributed to the inability to detect significant differences.

The current findings provide another reason for continued advocacy for caution regarding fluoroquinolone use among people at risk for active TB. The older-generation fluoroquinolones, particularly ciprofloxacin, generally have poorer bactericidal activity than the newer agents [9]. Ciprofloxacin has been associated with slower conversion rates and increased numbers of treatment failures and relapses in clinical studies, and it has been recommended not to use ciprofloxacin in the treatment of MDR-TB [1]. The newer fluoroquinolones, especially moxifloxacin, have shown potential for use in treating drug-susceptible TB as part of shorter first-line drug regimens [2]. Therefore, if exposure to the newer fluoroquinolones before TB diagnosis is a risk for selecting high-level fluoroquinolone resistance, inappropriate use of the newer agents could jeopardise the ability to use this important class of medications for anti-TB treatment. It is vital for clinicians to select specific fluoroquinolones for the appropriate infection.

We used the standard ofloxacin critical concentration [28] to classify *M. tuberculosis* isolates as fluoroquinolone-resistant or -susceptible and compared ofloxacin MIC values since cross-resistance occurs within the fluoroquinolone class [15,21]. One of the major limitations of the current study, however, is the classification of the specific fluoroquinolones. Although older- and newer-generation fluoroquinolones were distinguished based on clinical pharmacological parameters, important differences between specific fluoroquinolones exist. Particularly relevant to our findings, moxifloxacin has a C-8 methoxy side chain that structurally distinguishes it from levofloxacin, rendering broader antimicrobial properties and diminished efflux from *Streptococcus pneumoniae* [29]. On the other hand, although levofloxacin is structurally similar to ofloxacin in that it is its pharmacologically active enantiomer, the pharmacological profile of levofloxacin allows it to be classified with the newer fluoroquinolones [30]. By combining levofloxacin and moxifloxacin in our analysis, important differences between the two may have been obscured.

This study had several other limitations. First, the small case number limited the extent to which multivariate regression models could adjust for additional variables. However, it was still possible to adjust for potential confounding variables via multivariate analysis and to identify significant associations. Second, none of the fluoroquinolone exposure ascertainment methods were able to account for patient non-adherence. The TennCare pharmacy database only provided information about outpatient prescriptions and had programmatic changes in 2005 and 2006 that could have led to under-ascertainment of the number of patients who received fluoroquinolones. Specifically, we found that eight of the nine patients who were not exposed to fluoroquinolones and who had fluoroquinolone-resistant isolates had TB from 2002 to 2006. Since inpatient fluoroquinolone prescriptions were not captured during this time, it is possible that some of these patients were misclassified. In addition, we may not have captured all outpatient prescriptions. Although it

would be less likely, some of the patients who had fluoroquinolone-resistant TB isolates but did not have documented fluoroquinolone exposure could have been infected with a fluoroquinolone-resistant strain. Multiple sources of ascertainment provided broader assessment of fluoroquinolone exposure both from outpatient and inpatient settings after 2006. The fluoroquinolone exposure survey and in-home questionnaire relied on patient recall of prescriptions and could have been affected by recall bias. The clinic and hospital record review, however, provided more objective fluoroquinolone exposure assessments. Ideally, we would be able to study patients exposed only to older or only to newer fluoroquinolones, but this is unlikely to occur given the tendency toward using newer-generation agents. In addition, we were unable to draw any conclusions about reasons for fluoroquinolone prescription or specific medical diagnoses owing to limited availability of these data.

This study had several strengths. Notably, the case isolates were largely fluoroquinolone monoresistant, so that resistance to other antituberculous drugs did not interfere with the analysis. In addition, we had well documented, comprehensive fluoroquinolone exposure data that we were able to link to isolate resistance and mutation data.

Characterisation of older and newer fluoroquinolone exposure and selection of resistance mutations, both before TB diagnosis as in the current study and during treatment for TB, will hopefully contribute to the development of strategies to prevent the spread of drug-resistant TB. To our knowledge, this study is the first to suggest that clinical use of fluoroquinolones before *M. tuberculosis* diagnosis may place older agents lower in the mutant selection window than newer agents. In this setting, assumptions that antimicrobials with lower MIC or longer half-life will be better for preventing drug resistance may not necessarily be true. Particular attention to the appropriate use of the newer-generation fluoroquinolones is important as these agents are frequently used to treat MDR-TB and may eventually be incorporated into first-line treatment of drug-susceptible TB.

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Table 1

Demographic and clinical characteristics by exposure to fluoroquinolones (FQs)

Characteristic	Any FQ exposure (*n* = 40)	No FQ exposure (*n* = 35)	*P*-value
Age (years) [median (IQR)]	59 (41–79)	56 (42–72)	0.3
Sex [*n* (%)]			0.5
Male	23 (58)	23 (66)	
Female	17 (43)	12 (34)	
Black or African–American race	15 (38)	8 (23)	0.2
Hispanic or Latino ethnicity	0 (0)	5 (14)	0.01
Birthplace			0.4
US born	36 (90)	29 (83)	
Foreign born	4 (10)	6 (17)	
HIV seropositive (among those tested for HIV)	6/35 (17)	3/32 (9)	0.4
Resistance to other drugs			
Isoniazid	0/38 (0)	0/33 (0)	–
Rifampicin	0/39 (0)	0/33 (0)	–
Pyrazinamide	0/30 (0)	1/29 (3)	0.3
Ethambutol	1/39 (3)	1/33 (3)	0.9
Drug resistance to any of HRZE	1 (2)	2 (6)	0.5
MDR-TB	0 (0)	0 (0)	–

IQR, interquartile range; HIV, human immunodeficiency virus; HRZE, isoniazid, rifampicin, pyrazinamide and ethambutol; MDR-TB, multidrug-resistant tuberculosis.

Table 2

Fluoroquinolone (FQ) exposure characteristics by resistance or susceptibility to FQs

FQ exposure variable	FQ-susceptible (*N* = 50) [*n* (%)]	FQ-resistant (*N* = 25) [*n* (%)]	*P*-value
Any FQ exposure	24 (48)	16 (64)	0.2
Any exposure to specific FQs			
Older FQs [a]	11 (22)	4 (16)	0.5
Ofloxacin	1 (2)	0 (0)	0.5
Ciprofloxacin	10 (20)	4 (16)	0.7
Newer FQs [b]	17 (34)	14 (56)	0.07
Levofloxacin	14 (28)	11 (44)	0.2
Moxifloxacin	4 (8)	4 (16)	0.3
Gatifloxacin	1 (2)	1 (4)	0.6
Exposure to older versus newer			0.3
Exposure only to older FQs[a]	7 (14)	2 (8)	
Exposure only to newer FQs[b]	13 (26)	12 (48)	
Exposure to older and newer FQs[a,b]	4 (8)	2 (8)	
FQ unexposed	26 (52)	9 (36)	

[a]Older FQs include ofloxacin and ciprofloxacin.

[b]Newer FQs include levofloxacin, moxifloxacin and gatifloxacin.

Table 3

Association of duration of exposure to (A) newer fluoroquinolones (FQs) and (B) older FQs with minimum inhibitory concentration

(A) Newer FQs

	Adjusted OR (95% CI)	***P*-value**
Age	1.42 (0.70–2.88)	0.33
Female sex	0.58 (0.24–1.40)	0.23
Duration of only newer FQs [a]	1.79 (1.22–2.64)	<0.01

(B) Older FQs

	Adjusted OR (95% CI)	*P*-value
Age	1.38 (0.67–2.85)	0.38
Female sex	0.73 (0.30–1.74)	0.47
Duration of only older FQs [b]	0.94 (0.50–1.78)	0.86

OR, odds ratio; CI, confidence interval.

[a] Newer FQs include moxifloxacin, levofloxacin and gatifloxacin.

[b] Older FQs include ofloxacin and ciprofloxacin.

Table 4

Comparison of type of fluoroquinolone (FQ) exposure to mutations in *gyrA*[a]

	Older FQs only[b,c] (*N* = 9) [*n* (%)]	Newer FQs only[b,c] (*N* = 25) [*n* (%)]	*P*-value
gyrA codon 94	1 (11)	9 (36)	0.16
gyrA codons 90, 91, 94	1 (11)	11 (44)	0.08
QRDR *gyrA*	3 (33)	12 (48)	0.45
gyrA	8 (89)	24 (96)	0.44

QRDR, quinolone resistance-determining region (extends from codons 74 to 113 in *gyrA*).

[a] Mutations listed regardless of FQ susceptibility. Note that all mutations at codons 90, 91 or 94 occurred in FQ-resistant isolates.

[b] Newer FQs include moxifloxacin, levofloxacin and gatifloxacin; older FQs include ofloxacin and ciprofloxacin.

[c] Number of isolates with at least one mutation from designated location.