

# Fluoroquinolone Susceptibility among *Mycobacterium tuberculosis* Isolates from the United States and Canada

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**Background.** There is increasing interest in the possible role of new fluoroquinolone antibiotics for treatment of tuberculosis, but widespread use of fluoroquinolones for treatment of other bacterial infections may select for resistant strains of *Mycobacterium tuberculosis*.

**Methods.** We evaluated fluoroquinolone susceptibility using the proportion method (critical ciprofloxacin concentration for susceptibility testing, 2.0 µg/mL) in isolates obtained from patients enrolled in Tuberculosis Trial Consortium clinical trials during the period of 1995–2001 and in a referral sample of isolates sent to the Centers for Disease Control and Prevention (Atlanta, GA) during the period of 1996–2000 for additional testing, often because of drug resistance.

**Results.** Of the 1373 isolates from the clinical trials, 1324 (96%) were susceptible to isoniazid and rifampin; 2 (0.15%) of these isolates were also resistant to ciprofloxacin. Of the 1852 isolates from the referral sample, 603 (32.6%) were resistant to isoniazid and rifampin (i.e., multidrug resistant), 849 (45.7%) were resistant to ≥1 first-line drug but were not resistant to both isoniazid and rifampin, and 400 (21.6%) were susceptible to all first-line agents. Ciprofloxacin resistance was found in 33 (1.8%) of the referral-sample isolates. Most ciprofloxacin-resistant isolates (25 [75.8%]) were resistant to isoniazid and rifampin.

**Conclusions.** Despite widespread use of fluoroquinolones for treatment of common bacterial infections, resistance among clinical isolates of *M. tuberculosis* in the United States and Canada remains rare, occurring primarily among multidrug-resistant strains.

The fluoroquinolone antibiotics are the most promising new class of drugs for treatment of tuberculosis. Newer members of this class, such as levofloxacin, moxifloxacin, and gatifloxacin, are very active in vitro and in animal models of tuberculosis [1, 2]. There are now indications that these drugs may have potent sterilizing activity [3–5], in addition to indications that they may prevent resistance to other agents in a multidrug regimen. Clinical experience is limited, but the new fluoroquinolones, such as moxifloxacin, appear to improve

the outcome of treatment of multidrug-resistant tuberculosis [6]. Although formal studies with prolonged dosing are lacking, the fluoroquinolones appear to be well tolerated [7], even when administered for >12 months as part of treatment of multidrug-resistant tuberculosis [6]. As a result, clinical trials are underway to evaluate the activity of fluoroquinolones in the treatment of drug-susceptible tuberculosis.

The fluoroquinolones were approved for the treatment of a number of bacterial infections. Because of their broad spectrum of activity and favorable toxicity profile, short courses of fluoroquinolones are increasingly used to treat common bacterial infections, such as urinary and respiratory tract infections. In some areas of the world, resistance to fluoroquinolones is emerging among common pathogens, such as *Neisseria gonorrhoea* [8], *Escherichia coli* [9], and *Streptococcus pneumoniae* [10, 11]. Extensive use of fluoroquinolones

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for treatment of bacterial infections might result in primary fluoroquinolone-resistant tuberculosis. If primary resistance became common, this would negate the potential of fluoroquinolones to become part of first-line tuberculosis treatment. Therefore, we evaluated the rate of fluoroquinolone resistance among *Mycobacterium tuberculosis* isolates from the United States and Canada. Some of the results of this study have been previously reported in abstract form [12].

## METHODS

**Selection of isolates.** Two collections of *M. tuberculosis* isolates were available for study: (1) isolates recovered from patients enrolled in treatment studies of the Tuberculosis Trials Consortium (TBTC) [13–16], and (2) isolates sent for additional testing to the Mycobacteriology Laboratory of the Centers for Disease Control and Prevention (CDC; Atlanta, GA) from health departments around the United States.

The TBTC enrolls patients into treatment trials from 23 geographically dispersed sites around the United States and Canada. Isolates for this study were obtained from adult patients who enrolled in 4 clinical trials during the period of 1995–2001 (table 1). Three of these trials required that the patient's isolate be susceptible to isoniazid and rifampin; the fourth required resistance to isoniazid but susceptibility to rifampin. Two trials allowed enrollment of HIV-coinfected patients.

The Mycobacteriology Laboratory at the CDC receives a large number of *M. tuberculosis* isolates from local and state health departments in the United States for additional testing. One of the most common reasons for referral is the presence of resistance to first-line antituberculosis drugs, because many laboratories do not perform susceptibility tests for second-line agents. Other isolates are sent because of unusual growth characteristics or for genotyping.

The TBTC clinical trials from which these isolates were obtained were approved by institutional review boards at the CDC and at participating sites. Analysis of the referral isolates was performed with a data set containing no personal identifiers about the patients from whom the isolates were obtained.

**Laboratory methods.** All isolates were confirmed to be *M. tuberculosis* by conventional biochemical tests [17]. Fluoro-

quinolone susceptibility testing was performed with use of the proportion method with 2 µg/mL of ciprofloxacin. DNA was isolated from the *M. tuberculosis* strains as described elsewhere [18]. The 320–base pair *gyrA* gene was amplified using primers *gyrA*-1 (5'-CAGCTACATCGACTATGCCA-3') and *gyrA*-2 (5'-ATGAGGTACACCGAAGCCC-3') and a HotStart Taq amplification mix (Qiagen), as recommended by the manufacturer. Samples were incubated at 96°C for 15 min; amplified for 35 cycles at 96°C for 30 s, 30 cycles at 55°C for 30 s, and 30 cycles at 72°C for 30 s; incubated at 72°C for 7 min; and stored at 4°C. DNA sequencing was performed with the *gyrA*-1 and *gyrA*-2 primers and the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) in accordance with manufacturer's directions. The samples were analyzed using an ABI 373A/373XL automatic DNA sequencer (Global Medical Instrumentation).

**Data analysis.** To evaluate whether isolates recovered from patients enrolled in TBTC trials are representative of those recovered from patients with tuberculosis in the United States, we compared the demographic and clinical characteristics of TBTC patients with those of patients with tuberculosis in the United States during the period of 1995–2001. All analyses were performed using SAS software, version 6.12 (SAS Institute), and Epi Info software, version 6.04d (CDC).

## RESULTS

There were 1373 isolates available from TBTC studies. In general, patients enrolled in TBTC studies had characteristics similar to those of all patients with active tuberculosis in the United States during the same period (table 2). Nearly 40% of TBTC patients were born outside of the United States and Canada, and the most common countries of origin were similar to those of the overall population with tuberculosis. Because of the eligibility requirements of the largest trials from which these isolates were obtained, a lower proportion of TBTC patients had isolates that were resistant to isoniazid and/or rifampin than was true for the United States as a whole. Three isolates from TBTC patients had rifampin resistance found during retesting at the CDC laboratory (the patients were enrolled on the basis of tests showing rifampin susceptibility at a local laboratory).

**Table 1. Key enrollment criteria of Tuberculosis Trials Consortium (TBTC) trials from which *Mycobacterium tuberculosis* isolates were recovered.**

TBTC trial	Conventional agents for which susceptibility was required	Inclusion of HIV-infected patients	No. of isolates included in the study
22	Isoniazid, rifampin	Yes (70 patients)	1074
23	Rifampin (148 of 150 isolates were susceptible to isoniazid)	Yes: only HIV-infected patients were enrolled	150
24	Rifampin	No	34
25	Isoniazid, rifampin	No	154

**Table 2. Comparison of demographic and clinical characteristics of patients in Tuberculosis Trials Consortium (TBTC) trials with those of patients with active tuberculosis in the United States, 1995–2001.**

Characteristic	Patients in TBTC trials (n = 1373)	All US patients with tuberculosis, 1995–2001 (n = 93,233)
Age, mean years ± SD	43.6 ± 14.2	49.0 ± 20.2
Sex, % of patients		
Male	75	64
Female	25	36
Born outside of the United States or Canada	523 (38)	36,807 (40)
Country of birth outside of the United States or Canada <sup>a</sup>		
Mexico	189 (36)	8138 (22)
Vietnam	56 (11)	3966 (11)
The Philippines	49 (9)	4672 (13)
China	23 (4)	1962 (5)
Other	206 (39)	18,069 (49)
Race/ethnicity		
White	232 (17)	23,635 (25)
Black	575 (42)	30,888 (33)
Hispanic	340 (25)	19,147 (21)
Asian/Pacific Islander	179 (13)	18,084 (19)
Native American	42 (3)	1292 (1)
Other	5 (0.4)	NR
HIV seropositivity	201 (15)	11,207 (12)
Homelessness <sup>b</sup>	258 (19)	6323 (7)
Recent or current incarceration <sup>b</sup>	189 (14)	3554 (4)
Isolate with resistance to isoniazid	46 (3)	6894 (7)
Isolates with resistance to rifampin	3 (0.2)	1683 (2)

**NOTE.** Data are no. (%) of patients, unless otherwise indicated. NR, not reported.

<sup>a</sup> Percentages represent only persons born outside of the United States or Canada.

<sup>b</sup> The TBTC defined these as being present if they had occurred within the 5 years before tuberculosis diagnosis; the Centers for Disease Control and Prevention surveillance system used 1 year before TB diagnosis as the criterion.

Two (0.15%) of the 1373 isolates recovered from patients in TBTC trials were resistant to ciprofloxacin (1 isolate was also resistant to streptomycin). Both patients from whom these isolates were obtained were HIV infected but did not have advanced AIDS (CD4 cell counts, 221 and 468 cells/mm<sup>3</sup>). Therefore, the rate of primary fluoroquinolone resistance among isolates recovered from HIV-infected patients was 1% (2 of 201). Neither patient received a fluoroquinolone antibiotic during tuberculosis treatment, but there was no information about prior use of fluoroquinolones.

A total of 1852 isolates were referred to the Mycobacteriology Laboratory at the CDC during 1996–2000. No clinical data, demographic data, or treatment histories were available about the patients from whom these isolates were obtained. Most of the referral isolates (1452 [78.4%] of 1852) were drug resistant (table 3): 603 (32.6%) were resistant to isoniazid and rifampin (i.e., multidrug resistant), and 849 (45.7%) were resistant to

≥1 drug but not to both isoniazid and rifampin. Ciprofloxacin resistance was found in 33 (1.8%) of the 1852 referral sample isolates. Of the ciprofloxacin-resistant isolates, most (25 [75.8%] of 33) were in the multidrug-resistant group. Only 1 isolate had ciprofloxacin monoresistance.

There were 35 ciprofloxacin-resistant isolates from the TBTC and CDC mycobacteriology referral collections, 30 (86%) of which were available for additional testing. Twenty-six (87%) of the 30 isolates had mutations in the *gyrA* gene that have previously been associated with fluoroquinolone resistance (table 4). Four isolates had wild-type *gyrA* genes. These 4 isolates were confirmed to be ciprofloxacin resistant during retesting.

## DISCUSSION

With use of 2 collections of isolates, we found that fluoroquinolone resistance is uncommon among *M. tuberculosis*

**Table 3. Patterns of drug resistance among *Mycobacterium tuberculosis* isolates referred to the Mycobacteriology Laboratory at the Centers for Disease Control and Prevention, 1996–2000.**

Pattern of resistance	No. (%) of isolates	No. (%) of ciprofloxacin-resistance isolates
Susceptible to all first-line drugs <sup>a</sup>	400 (21.6)	1 (0.3)
Resistant to $\geq 1$ first-line drug but not to isoniazid and rifampin	849 (45.8)	7 (0.8)
Resistant to isoniazid and rifampin	603 (32.6)	25 (4.1)
Total	1852	33 (1.8)

<sup>a</sup> Isoniazid, rifampin, pyrazinamide, or ethambutol.

strains recovered from patients in the United States and Canada. The 2 collections of isolates included in this study are complementary. Because of eligibility requirements for recent clinical trials, most of the TBTC isolates were susceptible to isoniazid and rifampin, but they have the advantage of being otherwise representative of *M. tuberculosis* isolates in the United States. Conversely, the most common reason for referral of an isolate to the CDC laboratory is the presence of drug resistance, often multidrug resistance. Therefore, the consistent results from these 2 complementary collections of isolates provides strong evidence that *M. tuberculosis* resistance to fluoroquinolones is very uncommon in the United States and occurs primarily among isolates that are resistant to at least isoniazid and rifampin. Fluoroquinolones are commonly used for the treatment of multidrug-resistant tuberculosis, and resistance in these referral strains may represent selection of resistance during fluoroquinolone treatment. However, lack of clinical data regarding the patients from whom these isolates were obtained limits our understanding of fluoroquinolone resistance in multidrug-resistant strains.

There have been 2 prior studies of primary fluoroquinolone-resistant *M. tuberculosis* in the United States. Among 135 isolates collected during 1993–1995 from HIV-infected patients, the rate of resistance to levofloxacin was 0.7% [19], comparable to the rate of resistance among isolates recovered from HIV-infected patients in this study (1%). A recent small study in Baltimore, Maryland, found a higher rate of resistance (2 [4%] of 55 cases); both patients with primary fluoroquinolone resistance had been recently treated with a fluoroquinolone because their initial presentation with pulmonary tuberculosis was misdiagnosed as bacterial pneumonia [20]. Rates of primary fluoroquinolone-resistant tuberculosis were substantially higher in recent studies from Thailand (1.8% of 1738 cases) [21], India (3.6% of 1426) [22], Spain (6.1% of 213) [23], and The Philippines (18% of 100) [24]. Among patients who have received prior tuberculosis treatment, rates of fluoroquinolone resistance are higher—as high as 43% in

the study from The Philippines [24]—but this may represent the acquisition of resistance if a fluoroquinolone was used to treat tuberculosis due to strains that were resistant to the first-line drugs. Therefore, although our study shows that the rate of primary fluoroquinolone resistance is very low in the United States and Canada, this is not necessarily true in other areas of the world and may be changing with further selective pressure from fluoroquinolone use.

Mutations in the *gyrA* gene were the most common mechanism of fluoroquinolone resistance in *M. tuberculosis* in this and previous studies [19, 25, 26]. Isolates with *gyrA* mutations in this study had single mutations, which generally confer a 2–8-fold increase in the mean inhibitory concentration [27]. Isolates with  $\geq 2$  mutations in *gyrA*, which confer high-level fluoroquinolone resistance, have been selected in laboratory experiments [27] but were not identified in these clinical isolates. As has been documented in previous studies [25, 26], a minority of fluoroquinolone-resistant *M. tuberculosis* isolates do not have recognized mutations in the *gyrA* gene. The mechanism of fluoroquinolone resistance among isolates having wild-type *gyrA* has yet to be identified, but it may include a mutation in DNA *gyrB*, altered cell permeability, an efflux pump that decreases intracellular concentrations of the drug, or intracellular inactivation of the fluoroquinolone [28].

Our study has several limitations that should be noted. First, although patients who enrolled in the TBTC studies appeared to be representative of patients with tuberculosis in the United States, patients who decide to enroll in clinical trials may be different than patients who do not or cannot enroll. Whether factors associated with enrollment in a study might affect baseline resistance rates is not known. Second, our study assessed fluoroquinolone resistance among isolates recovered from patients with tuberculosis diagnosed during the period of 1995–2001. With continued fluoroquinolone use, the rate of primary resistance may be increasing. Third, we did not have information about prior fluoroquinolone use; thus, we could not evaluate the important question of the duration of fluoroquinolone exposure that commonly results in selection for a resistant strain. Finally, we only evaluated the susceptibility of isolates to ciprofloxacin, a fluoroquinolone that is less potent against *M. tuberculosis* than are newer members of this class.

**Table 4. Results of DNA sequencing of the *gyrA* gene in ciprofloxacin-resistant isolates of *Mycobacterium tuberculosis*.**

Codon	Wild-type amino acid	Amino acid coded in mutant isolate	No. (%) of isolates <sup>a</sup> (n = 30)
90	Ala	Val	4 (13)
94	Asp	Tyr, Gly, or Ala	22 (73)

<sup>a</sup> Four isolates had wild-type *gyrA* genes.

Although cross-resistance to the fluoroquinolones appears to be very common in *M. tuberculosis* [26, 29], it is possible that resistance rates of more-potent fluoroquinolones, such as moxifloxacin and gatifloxacin, are different than that we found for ciprofloxacin.

In summary, fluoroquinolone resistance is very uncommon among isolates from the United States and Canada and occurs primarily among multidrug-resistant isolates. These results support the development of clinical trials to evaluate the possible role of fluoroquinolones in the treatment of drug-susceptible tuberculosis. Additional studies will be needed to further evaluate rates of resistance in other parts of the world and to follow trends in fluoroquinolone resistance over time. The World Health Organization has developed a network of laboratories to conduct population-based studies of drug resistance in *M. tuberculosis* and is undertaking studies of primary fluoroquinolone resistance [30].

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