



Mutations in *gyrA* and *gyrB* among Fluoroquinolone- and Multidrug-resistant *Mycobacterium tuberculosis* Isolates

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Abstract

Background For multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB) isolates, it is unclear whether resistance-associated mutations outside the QRDR of *gyrA* and *gyrB* are associated with fluoroquinolone (FQ) resistance.

Materials/methods Drug susceptibility of the isolates was tested using modified proportional disk elution methods. DNA sequencing of the entire *gyrA* and *gyrB* genes were amplified with 8 primer sets. Genotyping (spoligotyping and 24-locus mycobacterial interspersed repetitive-unit-variable-number tandem-repeat (MIRU-VNTR) genotyping) was performed as previously described.

Results A total of 111 MDR-TB isolates were categorized into ofloxacin (OFX)-susceptible (minimum inhibitory concentrations, MIC ≤ 2 $\mu\text{g/ml}$), low- (MIC 4-8 $\mu\text{g/ml}$) and high-level (MIC ≥ 16 $\mu\text{g/ml}$) OFX-resistant isolates, and moxifloxacin (MFX)-susceptible (MIC ≤ 0.5 $\mu\text{g/ml}$), low- (MIC 1-2 $\mu\text{g/ml}$) and high-level (MIC ≥ 4 $\mu\text{g/ml}$) MFX-resistant isolates. Resistance-associated mutations inside the *gyrA* gene were found in 30.2% of OFX-susceptible, 72.5% and 72.2% of low- and high-level OFX-resistant isolates, and in 28.6% of MFX-susceptible, 58.1% and 83.9% of low- and high-level MFX-resistant isolates. Compared with OFX-susceptible, low- and high-level OFX-resistant isolates had a significantly higher prevalence of mutations at *gyrA* codons 88–94 (17.0%, 65.0% and 72.2%, respectively; $P < 0.001$) and a higher prevalence of the *gyrB* G512R mutation (0.0%, 2.5% and 16.7%, respectively; $P = 0.006$). Similarly, compared with MFX-susceptible, low- and high-level MFX-resistant isolates had a significantly higher prevalence of mutations at *gyrA* codons 88–94 (14.3%, 51.6% and 80.6%, respectively; $P < 0.001$) as well as *gyrB* G512R mutation (0.0%, 0.0% and 12.9%, respectively; $P = 0.011$). D94G and D94N mutations in *gyrA* and the G512R mutation in *gyrB* were correlated with high-level MFX resistance while the D94A mutation was associated with low-level MFX resistance. The prevalence of those resistance-associated mutations was higher among fluoroquinolone-susceptible East Asian (Beijing) and Indo-Oceanic strains than Euro-American strains.

Conclusion Our data imply that molecular techniques to detect FQ resistance may be less specific in areas with a high prevalence of FQ-resistant MDR-TB strains

Introduction

In this study, we evaluated the possible association between level of resistance to OFX and MFX and mutations in and outside of the QRDR of the *gyrA* and *gyrB* genes in MTB isolates.

Materials and methods

Selection of isolates. From 2006 to 2011, a total of 55 consecutive OFX-resistant MDR isolates and 56 OFX-susceptible MDR isolates were collected

DNA sequencing. The entire *gyrA* and *gyrB* genes were amplified with 8 primer sets.

Statistical analysis. The sequences from isolates were compared to the reference strain sequence and the proportions of resistant and susceptible isolates harboring specific mutations were compared using the Chi-square test or Fisher's exact test as appropriate.

TABLE 2 Mutations in the *gyrA* and *gyrB* genes and associated levels of ofloxacin and moxifloxacin resistance in multidrug-resistant *Mycobacterium tuberculosis*

Gene mutations (codon/nucleotide)	Ofloxacin				Moxifloxacin			
	Resistance				Resistance			
	Susceptible (n=53)	Low-level (n=40)	High-level (n=18)	P-value	Susceptible (n=49)	Low-level (n=31)	High-level (n=31)	P-value
<i>gyrA</i>								
Codon 88-94	9	26	13	<0.001	7	16	25	<0.001
G88C (Ggc/Tgc)	0	0	1	0.074	0	0	1	0.559
A90V (gCg/gTg)	2	5	0	0.112	3	4	0	0.106
S91P (Tcg/Ccg)	1	1	0	0.801	0	2	0	0.152
Codon 94	6	20	12	<0.001	4	10	24	<0.001
D94G (GAc/GGc)	2	11	4	0.002	2	3	12	<0.001
D94N (GAc/AAc)	0	4	5	0.001	0	2	7	0.001
D94A (GAc/GCc)	1	3	2	0.170	0	4	2	0.032
D94H (GAc/CAC)	1	2	0	0.752	1	1	1	1.000
D94V (GAc/GTc)	2	0	0	0.653	1	0	1	1.000
D94Y (GAc/TAc)	0	0	1	0.162	0	0	1	0.559
A132S (Gcc/Tcc)	0	1	1	0.143	0	0	2	0.152
P190L (cCg/cTg)	0	1	0	0.523	0	0	1	0.559
A288D (gCc/gAc)	0	1	0	0.523	1	0	0	1.000
T335A (Acc/Gcc)	1	0	0	1.000	1	0	0	1.000
A384V (gCa/gTa)	7	5	2	1.000	6	4	4	1.000
<i>gyrB</i>								
A227D (gCc/gAc)	1	0	0	1.000	1	0	0	1.000
H244N (Cac/Aac)	0	1	1	0.143	0	1	1	0.310
M291I (atG/atC)	7	4	2	0.923	6	3	4	1.000
A403S (Gcg/Tcg)	1	2	0	0.752	0	3	0	0.041
D461N (Gac/Aac)	1	0	0	1.000	1	0	0	1.000
I485V (Atc/Gtc)	0	1	0	0.523	0	1	0	0.559
Any codon 499	1	1	2	1.000	1	1	2	0.811
N499D (AAc/GAc)	1	1	1	0.565	1	1	1	1.000
N499T (AAc/ACc)	0	0	1	0.162	0	0	1	0.564
A504T (Gcg/ACg)	0	0	1	0.162	0	0	1	0.564
A508S (Gcg/Tcg)	1	0	0	1.000	0	1	0	0.559
G512R (Ggg/Agg)	0	1	3	0.006	0	0	4	0.011
Wild type	34	9	3	<0.001	33	10	3	<0.001

TABLE 1. Minimum inhibitory concentrations of ofloxacin and moxifloxacin established for multidrug-resistant *Mycobacterium tuberculosis* with resistance-associated mutations in the *gyrA* and *gyrB* genes

Gene mutations (codon/nucleotide)	No.	MIC ($\mu\text{g/ml}$) of ofloxacin										MIC ($\mu\text{g/ml}$) of moxifloxacin									
		0.25	0.5	1	2	4	8	16	32	>32	0.06	0.12	0.25	0.5	1	2	4	8	>8		
<i>gyrA</i>																					
G88C (Ggc/Tgc)	1																			1	
A90V (gCg/gTg)	4	1			1	2				1	1			1	1					1	
A90V (gCg/gTg)	1				1																
A90V (gCg/gTg) & A384V (gCa/gTa)	2		1																		
S91P (Tcg/Ccg)	2	1				1									2						
D94G (GAc/GGc)	13		1	1		1	7	2	1		1	1			3	6	2				
D94G (GAc/GGc)	1					1															
D94G (GAc/GGc) & P190L (cCg/cTg)	1						1														
D94G (GAc/GGc) & A384V (gCa/gTa)	2					1	1													2	
D94N (GAc/AAc)	6						3	3										1	3	1	1
D94N (GAc/AAc)	1							1													
D94N (GAc/AAc) & A132S (Gcc/Tcc)	1							1													1
D94N (GAc/AAc) & A132S (Gcc/Tcc)	1								1												
D94A (GAc/GCc)	1														1						
D94A (GAc/GCc)	2					1	1								1	1					
D94A (GAc/GCc)	1																				1
D94A (GAc/GCc) & A384V (gCa/gTa)	1							1													
D94A (GAc/GCc) & A384V (gCa/gTa)	1								1												1
D94V (GAc/GTc)	2			1		1															1
D94H (GAc/CAC)	3					1	2				1				1	1					
D94Y (GAc/TAc)	1							1													
A288D (gCc/gAc)	1										1										
T335A (Acc/Gcc)	1	1									1										
A384V (gCa/gTa)	8	1		1	4		2				1	3	1	2	1						
D461N (Gac/Aac)	1	1									1										
I485V (Atc/Gtc)	1					1								1							
N499D (AAc/GAc)	3					1	1							1	1	1					
A508S (Gcg/Tcg)	1																				1
G512R (Ggg/Agg)	1																				1
Total	111	10	13	15	15	14	26	11	3	4	2	20	17	10	15	16	17	9	5		

TABLE 3 Performance of mutation detection (*gyrA* codon 88-94 or *gyrB* G512R) for identification of ofloxacin and moxifloxacin resistance (low- and high-level) in different strains of multidrug-resistant *Mycobacterium tuberculosis*

Agent	Strain	Number of isolates				Performance % (95% confidence interval)				
		Resistant		Susceptible		Sensitivity	Specificity	LR+	LR-	Accuracy
		Mutation positive	Mutation negative	Mutation positive	Mutation negative					
Ofloxacin	All	40	18	9	44	69.0 (55.5-80.5)	83.0 (70.2-91.9)	4.1 (2.2-7.5)	0.4 (0.3-0.6)	75.7 (66.6-83.3)
	East Asian (Beijing)	18	7	5	24	72.0 (50.6-87.9)	82.8 (64.2-94.2)	4.2 (1.8-9.6)	0.3 (0.2-0.6)	77.8 (64.4-88.0)
	Euro-American	10	6	0	9	62.5 (35.4-84.8)	100.0 (66.4-100.0)	>10.0	0.4 (0.2-0.7)	76.0 (54.9-90.6)
	Indo-Oceanic	10	5	4	11	66.7 (38.4-88.2)	73.3 (44.9-92.2)	2.5 (1.0-6.2)	0.5 (0.2-1.0)	70.0 (50.6-85.3)
	Moxifloxacin	All	42	20	7	42	67.7 (54.7-79.1)	85.7 (72.8-94.1)	4.7 (2.3-9.6)	0.4 (0.3-0.5)
Moxifloxacin	East Asian (Beijing)	19	10	4	21	65.5 (45.7-82.1)	84.0 (63.9-95.5)	4.1 (1.6-10.4)	0.4 (0.2-0.7)	74.1 (60.3-85.0)
	Euro-American	10	5	0	10	66.7 (38.4-88.2)	100.0 (69.2-100.0)	>10.0	0.3 (0.2-0.7)	80.0 (59.3-93.2)
	Indo-Oceanic	10	5	4	11	66.7 (38.4-88.2)	73.3 (44.9-92.2)	2.5 (1.0-6.2)	0.5 (0.2-1.0)	70.0 (50.6-85.3)

LR+: Positive likelihood ratio. LR-: Negative likelihood ratio