Published in final edited form as:

Int J Tuberc Lung Dis. 2015 October 1; 19(10): 1227–1229. doi:10.5588/ijtld.14.0277.

Determination of MICs of Levofloxacin for *Mycobacterium tuberculosis* with *gyrA* Mutations

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Summary

The purpose of the present study was to correlate *gyrA* mutations found within in *Mycobacterium tuberculosis* (*MTB*) isolates by Genotype MTBDRs_l assay with Minimum Inhibitory Concentrations (MICs) of the fluoroquinolone levofloxacin (LVX). Of the 123 archived clinical *MTB* isolates evaluated, 93 isolates had an Ala90Val, Ser91Pro, Asp94Ala, Asn/Tyr, Gly or His mutation and 30 were wild type. Phenotypically, *gyrA* mutations with Ala90Val, Ser91Pro or Asp94Ala showed a low level of resistance to LVX while Asp94Asn/Tyr, Asp94Gly or Asp94His mutations demonstrated a high level of resistance.

Keywords

Level of resistance; Mutations; MTB

Fluoroquinolones (FQs) are extensively used in chemotherapy against multidrug-resistant (MDR) tuberculosis (TB). However, the development of FQ-resistance in TB is due to overuse of ofloxacin (OFX), ciprofloxacin and levofloxacin (LVX) in treatment of undiagnosed respiratory infections.¹ Additionally, FQ-resistance has now been observed in isoniazid (INH) and rifampicin (RIF)-susceptible strains isolated from newly diagnosed TB patients.^{2, 3}

Resistance to the FQs is attributable to mutations (single nucleotide polymorphisms, or SNPs) in the *gyrA* and *gyrB* genes, which encode the respective subunits of the DNA topoisomerase gyrase.⁴ The most common FQ resistance-conferring mutations occur in the *gyrA* gene and include Ala90Val, Asp94 (Gly, Ala, His, Asn or Tyr) and Ser91Pro.^{4,5,6} The Gly88Cys mutation is less frequently found.⁵ In order to build upon our knowledge of the fluoroquinolone compounds available for TB treatment, we performed LVX Minimum

Inhibitory Concentrations (MICs) for 123 *Mycobacterium tuberculosis* (MTB) isolates that were characterized in our earlier study.⁷ We correlated LVX MICs with specific *gyrA* mutations found via the Genotype MTBDR_{sl} assay to determine whether these commonly occurring mutations were associated with different levels of LVX resistance.

Methods

One hundred twenty-three archived isolates were selected for which both Genotype MTBDR_{sl} assay and drug susceptibility testing (DST) at the WHO approved critical concentrations had been performed previously. Written consent was waived for all participants as the study was carried out on archived isolates. Quantitative DST was performed via standard methods as per manufacturer instructions (Becton Dickinson Diagnostic System, Sparks, MD).⁸ A stock solution of LVX was prepared by dissolving the drug in 0.1N NaOH. The drug solution was then filtered, further diluted in distilled water, and stored at –80°C for up to 6 months. A critical concentration of 1.5µg/ml was used for LVX, in accordance with WHO recommendations.⁹ For estimation of LVX MICs, two concentrations below the critical concentration (0.38 and 0.75µg/ml), and three concentrations above the critical concentration (3.0, 6.0 and 12.0µg/ml), were utilized. Genotype MTBDR_{sl} assay was performed as per manufacturer instructions (Hain Lifescience, Nehren, Germany).¹⁰ Thirty representative isolates of the 123 MTB isolates were sequence-confirmed by PSQ. A Kruskal-Wallis statistical test (GraphPad Prism 6, one-way ANOVA) was performed.

Results

The overall agreement between Genotype MTBDR_{sl} and phenotypic test results was 93%. Disagreement was the result of isolates with *gyrA* mutations that had an MIC of 1.5, as all wild-type isolates had MICs <1.5. MIC testing results for the various *gyrA* mutations identified via the MTBDR_{sl} assay are summarized in Table 1. Thirty representative isolates of the 123 MTB isolates were sequence confirmed by PSQ. These isolates were selected so as to cover all the *gyrA* mutations identified by the Genotype MTBDR_{sl} assay. Four isolates with wild type characterization were also pyrosequenced. The Kruskal-Wallis test statistic was 58.44 with a p-value of <0.0001, suggesting that the levels of phenotypic resistance to LVX were significantly associated with specific *gyrA* mutations. The Kruskal –Wallis test results reveal that a low level of resistance to LVX was seen for isolates with Ala90Val, Ser91Pro or Asp94Ala mutations, and a high level of resistance was seen with isolates harboring Asp94Asn/Tyr, Asp94Gly or Asp94His mutations.

Discussion & Conclusion

There was a good correlation between the various *gyrA* mutations observed by Genotype MTBDR_{sl} assay and the levels of phenotypic resistance seen to LVX. *gyrA* mutations associated with FQ resistance were found in 93 of the 123 MTB isolates evaluated. In these FQ-resistant isolates, we observed seven unique mutations: Ala90Val, Ser91Pro, Asp94Ala, Asp94Asn, Asp94Tyr, Asp94Gly and Asp94His. A significant correlation was found between the LVX MICs and the position of the *gyrA* mutations. A dosing of LVX at 500mg

daily yields a peak serum concentration (C_{max}) of 6.21 µg/ml and an AUC₂₄ value of 44.8 µg·h/ml, resulting in a C_{max}/MIC of 5-7 and an AUC₂₄/MIC ratio of 40-50.⁴

In the present study, 100% (25/25) of isolates with the mutation Ala90Val had an MIC at or above the critical concentration of 1.5 µg/ml. Similarly, 100% (6/6) of isolates with a Ser91Pro mutation showed an MIC of 1.5-3.0 µg/ml. A previous study reported similar findings, with an MIC of 1.0 µg/ml for the mutations Ala90Val, Ser91Pro and Asp94Ala.¹¹ MICs ranging from 3.0-12.0 µg/ml for LVX were seen in 100% (42/42) of isolates with Asp94Gly mutations, in accordance with other studies reporting 60% (12/20) of the isolates with the mutation having an MIC of 10.0 µg/ml.¹¹ Isolates with Asp94Tyr and Asp94His showed an MIC₉₀ of 6.0 µg/ml. Similar results were found in earlier study with MIC of 4 µg/ml.⁵ For isolates with Ala90Val, Ser91Pro or Asp94Ala mutations, MICs were below the peak serum concentration (MIC₉₀ = 3 µg/ml or 1.5 µg/ml), whereas Asp94Asn/Tyr, Gly or His mutations had MICs at or above the peak serum concentration (MIC₉₀ = 6 µg/ml). Judged by MIC₉₀, mutations at Asp94Asn/Tyr, Gly or His were linked to a higher level of resistance to LVX compared to mutations at Ala90Val, Ser91Pro or Asp94Ala. These results were similar to those MIC levels previously reported for these isolates against other FQ compounds,⁷ suggesting that clinical *MTB* isolates with *gyrA* mutations in the 90 and 91 codons have the potential to be treated with standard or increased LVX dosing. Small differences discovered between the different FQ MIC findings, meanwhile, may have significant clinical implications in deciding how, and with what FQ compounds, to best treat different infections.

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Table 1

Levofloxacin MICs for genetically wild-type isolates and isolates determined to harbor resistance-associated mutations in the *gyrA* gene

Nucleotide changes	Genotype MTBDRs/	Mutation	Total No. (%)	No. Isolates	MIC (µg/ml)	MIC ₅₀ (µg/ml)	MIC ₉₀ (µg/ml)
GCG-GTG	MUT1	Ala90Val	25 (26.9)	20	3.0	3.0	3.0
				5	1.5		
TGC-CCG	MUT2	Ser91Pro	6 (6.5)	5	3.0	3.0	3.0
				1	1.5		
GAC-GCC	MUT3A	Asp94Ala	4 (4.3)	3	1.5	1.5	1.5
				1	6.0		
GAC-AAC/TAC	MUT3B	Asp94Asn/Tyr	13 (13.9)	10	6.0	6.0	6.0
				2	3.0		
				1	12		
GAC-GGC	MUT3C	Asp94Gly	42 (45.2)	34	6.0	6.0	6.0
				6	3.0		
				2	12		
GAC-CAC	MUT3D	Asp94His	3 (3.2)	1	3.0	6.0	6.0
				2	6.0		
WT	WT	WT	30	25	0.38	-	-
				5	0.75		