

**Genetic Characterization of
Rifampin-Resistant *Mycobacterium tuberculosis*
Isolates from the Research Institute for Tropical
Medicine**

A Thesis Submitted to the
Faculty of the Graduate School of the
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ABSTRACT

Rifampin plays a key component of the first line antitubercular drugs. However, resistance to rifampin in tuberculosis is gaining a foothold in the Philippines. Studies show that resistance to rifampin involves mutation in the *rpoB* gene which encodes the β subunit of the RNA polymerase. In this pilot study, DNA sequences of a 305-bp fragment of the *rpoB* gene were determined from 40 clinical isolates of *Mycobacterium tuberculosis* where 22 rifampin-resistant and 18 rifampin-susceptible isolates were obtained. All of the rifampin-susceptible isolates showed no mutations in the *rpoB* gene fragment, hi contrast, 20 out of 22 (90%) rifampin-resistant isolates showed missense mutations. Two isolates showed 2-step point mutations each and 1 isolate had a point mutation and a deletion. The rest showed one-step point mutations. The missense mutations reported here were similar to those reported by Telenti et al (Lancet 1993; 341:647-50), and Williams et al (Antimicrob. Agents Chemother. 1994; 38:2380-86). However, the discovery of an isolate with a point mutation and a deletion has not been reported elsewhere and is, therefore, considered novel. Because the study was limited to the analysis of mutations in the Cluster I region, it is probable that the 2 rifampin resistant isolates without mutations could have substitutions in other clusters of the *rpoB* gene.

With the number of rifampin-resistant cases on the rise, it is important to monitor the development and spread of rifampin-resistant isolates to enable clinicians to prescribe the appropriate antitubercular drug. The discovery of the mutant *rpoB* gene has led to the development of a molecular approach for the detection of rifampin resistant isolates from clinical specimens. This study utilizes the Polymerase Chain Reaction-Heteroduplex Formation (PCR-HDF), a molecular approach, to demonstrate for the first time, the utility of this tool for the rapid and accurate detection of rifampin-resistant *M. tuberculosis* in the Philippines.

TABLE OF CONTENTS

Title Page	i
Approval Sheet	ii
Abstract	iii
Dedication	v
Acknowledgments	vi
Table of Contents	viii
List of Figures	x
List of Tables	xi
CHAPTER	PAGE
I. The Problem and Its Background	
A. Introduction	1
B. Statement of the Problem	3
C. Significance of the Study	4
D. Theoretical Framework	5
E. Scope and Limitations	8
F. Definition of Terms	9
II. Review of Related Literature and Studies	
A. <u>Mycobacterium tuberculosis</u> and Its Disease	
1. Pathogenesis of Tuberculosis	13
2. Diagnosis of Tuberculosis	17
3. Treatment of Tuberculosis	20

UNIVERSITY OF SANTO TOMAS GRADUATE SCHOOL		PAGE
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B. Mechanism of Antimycobacterial Resistance	24
C. Rifampin	28
D. The <i>rpoB</i> gene and Rifampin Resistance	30
E. Polymerase Chain Reaction	33
F. Cycle Sequencing	36
G. Molecular Strategies Used to Detect Mutations In Genes Associated with Antimicrobial Resistance in Mycobacteria	38
III. Research Methodology	
Part I. Identification of <i>rpoB</i> Mutations among Rifampin Resistant <u><i>M. tuberculosis</i></u> isolates	
A. Extraction of Mycobacterial DNA	42
B. Amplification of the 305 bp fragment of the <i>rpoB</i> gene	43
C. DNA Sequencing	50
Part II. Optimization of conditions For Rapid Screening by Heteroduplex Formation	60
IV. Presentations of Findings, Analysis and Interpretation	
A. Characterization of Mutations in the <i>rpoB</i> gene	62
B. Heteroduplex Formation	69
V. Summary, Conclusions and Recommendations	
A. Conclusion	74
B. Summary	77
C. Recommendations	78
VI. Bibliography	81
VII. Appendices	91
VIII. Curriculum Vitae	112

List of Figures

	PAGE
1. The <i>rpoB</i> gene of <u>E.coli</u>	5
2. Summary of <i>rpoB</i> Mutations	6
3. Steps in the Pathogenesis of Tuberculosis	15
4. Structure of Rifampin	28
5. Thermocyclers Used in the Study	43
6. Gel Chamber for Agarose Gel Electrophoresis	46
7. UV Transilluminator with Camera	46
8. Graphical Representation of PCR	47
9. Minicolumn Assembly	48
10. DNA Sequencing Set-Up	56
11. Viewing The Plate on a White Light Box	59
12. PCR Product	63
13. Purified PCR Product	63
14. Silver Stained Sequencing Gel	63
15. DNA Sequences That Showed Mutations	64
16. PCR-HDF Results	71
17. Steps and Time Factor in PCR to PCR-HDF	73

List of Tables

	PAGE
1. Methods for Reporting Numbers of Acid Fast-Bacilli Observed in Stained Smears	20
2. Complex Alleles of <i>rpoB</i> found in Rifampin-Resistant Isolates	32
3. Summarized Description of PCR Components	35
4. Mutational Analysis: Results from Sequencing	65
5. Summary of Mutations	66
6. Heteroduplex Formation Results	70

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