بسم الله الرحمن الرحيم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

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جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

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MONA MAGHRABY

Molecular Study on Gene mutations associated with Antibiotic Resistance of *Mycobacterium tuberculosis*

Thesis

Submitted for partial fulfillment of the master's degree in Science (Microbiology)

Submitted By

Amal Mohamed Ali Hosny

(B. Sc degree in Microbiology)

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Approval Sheet

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Declaration

This dissertation has not been previously submitted for any degree at this or at any other university

Amal Mohamed Ali Hosny

Dedication

This work is dedicated to my beloved family and friends. I am truly thankful for your endless support and encouragement throughout this journey.

Amal Mohamed Ali Hosny

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Amal Mohamed Ali Hosny

List of Abbreviations

AC	Amplification Control Zone
AFB	Acid-Fast Bacilli
AM-A and AM-B	Amplification Mixes A And B
Bp	Base pair
CC	Conjugate Control Zone
CON-C	Conjugate Concentrate
DEN	Denaturation Solution
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide Triphosphate
DOTS	Directly Observed Treatment Short Course
DST	Drug-Susceptibility Testing

HYB	Hybridization Buffer
INH	Isoniazid
LJ	Lowenstein–Jensen
LSP	Large Sequence Polymorphisms
MDR-TB	Multidrug Resistant Tuberculosis
MIC	Minimum Inhibitory Concentration
MTBC	*Mycobacterium Tuberculosis* Complex
MUT	Mutant
NAAT	Nucleic Acid Amplification Test
NTMs	Non-Tuberculous Mycobacteria
R	Resistant
RDs	Regions of Difference
RFLP	Restriction Fragment Length Polymorphisms

RIF	Rifampicin
RIN	Rinse Solution
RNA	Ribonucleic acid
S	Sensitive
SNPs	Single Nucleotide Polymorphisms
STR	Stringent Wash Solution
SUB-C	Substrate Concentrate
SUB-D	Substrate Buffer
WT	Wild Type

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ABSTRACT

Amal Mohamed Ali Hosny

Molecular Study on Gene mutations associated with Antibiotic Resistance of Mycobacterium tuberculosis

Master's degree in Microbiology, Faculty of Science, Ain Shams University

In response to the huge number of people who die yearly due tuberculosis and the emergence of multidrug resistant (MDR) M. tuberculosis, accurate and rapid detection of this resistance can improve the situation. Relapsed patients in the current work represented significant percentages among rifampicin and isoniazid resistant isolates compared to other risk factors. Two molecular techniques (Genotype MTBDRplus assay and specific gene sequencing were used to detect associated mutations in TB drug resistant isolates. The genotypic profile of Multi-drug resistant (MDR) isolates showed missing of katG wild type 1 (WT1) band. Eighty percent of isoniazid mono-resistant isolates, showed katG MUT1, 20% showed katG MUT1 and inhA MUT1, 20% showed only inhA MUT1. The molecular techniques partly predicted the level of antibiotic resistance associated with katG and/or inhA gene mutations (for isoniazid) and rpoB gene mutation (for rifampicin). MTBDRplus could clearly detect rifampicin resistance among 66.7% of MDR isolates that showed mutation band rpoB MUT3 while 33.3% of them were considered as unknown, while 100% of mono-isoniazid resistant strains were detected. A mono-resistant rifampicin isolate did not show rifampicin mutation bands by Genotype MTBDRplus assay, but it showed unexpected mutation in codon 531 of rpoB by DNA sequence

analysis, it can be considered as heteroresistant strain. Gene sequencing could detect resistance associated mutations mainly in codon 315 (katG gene), position -15 (inhA gene) for isoniazid resistance and codon 531 (rpoB gene) for rifampicin resistance. The molecular techniques are able initially to predict rifampicin and isoniazid drug resistance and also level of resistance, but combination between molecular and phenotypic methods still recommended. Heteroresistance pattern and mixed infection with additional non-tuberculous mycobacteria are still considerable challenges. Effort should continue to explore more sensitive, rapid and specific molecular techniques for detection of resistance in M. tuberculosis, which will also improve the treatment opportunities.