شبكة المعلومات الجامعية

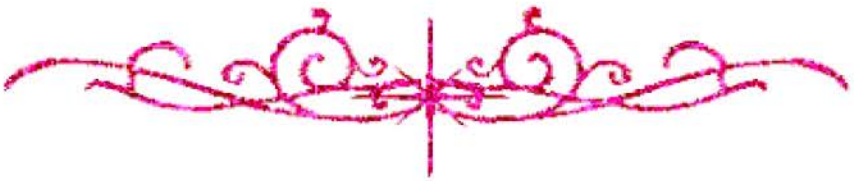
بسم الله الرحمن الرحيم

HOSSAM MAGHRABY

شبكة المعلومات الجامعية

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم



HOSSAM MAGHRABY

شبكة المعلومات الجامعية

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها

علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات

يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار

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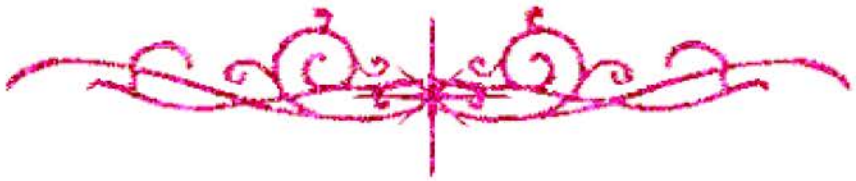
شبكة المعلومات الجامعية

بعض الوثائق الأصلية تالفة



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شبكة المعلومات الجامعية

بالرسالة صفحات

لم ترد بالأصل

HOSSAM MAGHRABY

Identification of Multidrug-Resistant *Mycobacterium tuberculosis*; Comparison of The Standard Susceptibility and The DNA-Based Methods

Thesis
Submitted in Partial Fulfillment of
M.D. degree in Microbiology

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بسم الله الرحمن الرحيم

قالوا سبحانك لا علم لنا إلا ما

علمتنا إنك أنت العليم الحكيم

صدق الله العظيم

(٣٢/البقره)

This work is dedicated to

My parents, my husband who suffered a lot during this work and my son "HAZEM".

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Abstract

Tuberculosis (TB), though curable, still remains a major killer disease worldwide. One of the most alarming trends concerning tuberculosis is the emergence of drug-resistant *M.tuberculosis* strains, which has become a worldwide health care problem (Pablos-Mendez *et al.*, 1998).

The aim of this study was to compare the agar proportion and SSCP methods for the detection of multiple drug resistance (MDR) in *M.tuberculosis*. A second goal was to characterize the mutations in *rpoB* and *katG* genes associated with RIF and INH resistance by DNA sequencing.

This study was conducted on 40 MTB isolates collected from smear-positive pulmonary tuberculosis patients from Ismailia and Suez Chest Hospitals. Drug susceptibility testing was performed on the MTB isolates against 4 anti-tuberculosis drugs (INH, RIF, SM and EMB) in concentrations of 0.2, 40, 8, 2 μg/ml respectively by the L-J agar proportion method. The highest rate of resistance was that for SM (47.5%) while the lowest was for EMB (25%). The rate of MDR (resistance to both INH and RIF) was 35%.

SSCP detected 13 INH resistant isolates (32.5%) while it detected 12 (30%) RIF resistant ones out of 40 MTB isolates. When comparing the results of SSCP to those of the agar proportion method (the gold standard), we found that its sensitivity was 72.2% and 80% when testing for *katG* and *rpoB* mutations respectively, its specificity was 100% and the 2 tests showed moderate to good agreement (Kappa= 0.74 and 0.83).

As regards the type of mutations and the resulting amino acid substitutions associated with drug resistance, DNA sequencing revealed that 13 of 14 INH-resistant isolates (92.8%) had mutations in *katG*; 12 had a $S_{315}T$ amino acid substitution and the remaining one isolate had a $D_{329}A$ substitution. Of the 13 RIF-resistant isolates, shown to have mutations in *rpoB* by sequencing, 10 (77%) had a $S_{531}L$ substitution and 3 isolates (23%) had $H_{526}Y$, $H_{526}L$ and $S_{531}W$ substitutions.

The sensitivity of SSCP was ~75% when compared to the agar proportion method and its specificity was 100%. This suggests that

SSCP can be used for rapid screening of a large number of isolates for their susceptibility but it cannot be used as a conclusive diagnostic method and SSCP results must be confirmed by conventional agar testing.