

## الآية



( رَبِّ أَوْزِعْنِي أَنْ أَشْكُرَ نِعْمَتَكَ الَّتِي أَنْعَمْتَ عَلَيَّ  
وَعَلَى وَالِدَيَّ وَأَنْ أَعْمَلَ صَالِحاً تَرْضَاهُ وَأَدْخِلْنِي  
بِرَحْمَتِكَ فِي عِبَادِكَ الصَّالِحِينَ }

صدق الله العظيم

سورة النمل ، الآية (19)

# *Dedication*

*I would like to dedicate this research  
to all those who always have been there  
for me.*

*To to the souls of my Father and mother*

*To my wife and son..*

*With much love ....*

Isam

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## Abstract

The spread of multidrug-resistant (MDR) strains of *Mycobacterium tuberculosis* has become a major public health concern since these bacteria often cause incurable disease, even when expensive second- and third-line drugs are available.

This study aimed to identify *M. tuberculosis* among suspected tuberculous patients in Khartoum and Gazeera States by using conventional methods also to identify rifampicin resistance *M. tuberculosis* by amplifying *rpoB* gene, using polymerase chain reaction (PCR). Moreover, it aimed to detect *M. tuberculosis* from direct sputum by PCR.

Out of 228 of sputum samples, 128 and 100 were collected from suspected tuberculosis patients in Khartoum and Gezeera states respectively. Smears were made stained directly using Ziehl-Neelsen (ZN) stain. The results showed that, among Khartuom state suspected patients, 36 (21.7%) were positive for acid fast bacilli (AFB) while 92 (78.3) were negative, and among Gazeera state suspected patients 23(23%) were positive while 77 (77%) were negative. All sputum samples from Khartuom were inoculated on Lowenstein Jensen (LJ) medium and incubated aerobically at 37°C, the isolates showed obvious growth in 46 (36%) whereas 82 (64%) showed no growth, while samples from Gazeera were suspected to direct to PCR.

Selected biochemical tests were performed to all *Mycobacterium tuberculosis* complex (MTC) isolates of Khartuom state, the results revealed that all isolates were sensitive to Para-nitro benzoic acid (growth was inhibited by PNB), resistant to thiophene - 2 - carboxylic acid

hydrazide (TCH), positive for nitrate reduction and were catalase negative at 68°C.

All the forty six isolates that showed typical growth of MTC on LJ medium were subjected to PCR to amplify IS *6110* gene .The results indicate clearly that all isolates showed 123bp bands for IS6110 gene, Drug sensitivity tests were performed to all isolates, the results showed that 26(20.3%) as MDR-TB, 16 (34.8%) as sensitive to rifampicin, Isoniazid, Ethambutol and streptomycin, 2 (4.4%) as resistance to streptomycin, and 2(4.4%) as triple resistance to Isoniazid, Ethambutol and streptomycin.

The twenty six resistant isolates were subjected to PCR searching for rifampicin resistance gene (*rpoB*) with band equal to 193bp in size, the results showed existence of this band in 20.3% of the mycobacterium tuberculosis isolates, of them 86.7% had resistance to *rpoB* gene.

Regarding Gazeera state, out of 74 isolates the resistant strains were 25 (33%) only 19 (19%) gave band typical in size to the target gene *rpoB* (193bp) as indicated by standard DNA ladder for the present of rifampicin resistant gene, of them 76% had resistance to *rpoB* gene.

Due to the low sensitivity of ZN technique and the long time required to conduct drug susceptibility test (DST) through conventional method, the results concluded the PCR is a valuable, rapid and sensitive technique which can replace conventional method, and PCR is also useful when taken directly from sputum.

The study recommended that, PCR assay could be introduced as a diagnostic tool for tuberculosis, and recommended to support the development of new tools and enable their timely and effective use.

## مستخلص الرسالة

بات انتشار المنفطرة السلية متعددة المقاومة للادوية من الشواغل الرئيسية للصحة العامة حيث ان هذه البكتيريا غالبا ما تسبب مرض عضال، بتوفر الادوية البديلة باهظة الثمن. هدفت هذه الدراسة للتعرف علي المنفطرة السلية بين المرضى المشتبه إصابتهم كل من ولاية الخرطوم و ولاية الجزيرة ، باستخدام الطرق التقليدية، والتعرف علي الجين (rpoB) المقاوم للمضاد ريفامبيسين السل متعدد المقاومة للادوية باستخدام تفاعل البلمرة التسلسلي والذي ا أيضاً لعزل المنفطرة السلية مباشرة من كهدف اخر.

من بين 228 من المرضى المشتبه إصابتهم بمرض السل تم جمع عدد مئة وثمان وعشرون عينة من الخرطوم ومائة من الجزيرة.

ZN. أظهرت النتائج أنه من بين عينات ولاية الخرطوم 36 (21%) كانت موجبة للعصيات المقاومة للحمض، بينما 92 (78.3%) كانت سالبة. ولدى ولاية الجزيرة 23 (23%) كانت موجبة للعصيات المقاومة للحمض بينما 77 (77%) كانت سالبة.

فيما يخص المجموعة ولاية الخرطوم 46 (36) كانت نتائج موجبة لتزريع المنفطرة السلية في وسط ليونستين جنسن عند درجة حرارة 37°C و 82 (64) أظهرت . وقد خضعت العينات من ولاية الجزيرة مباشرة لتفاعل البلمرة التسلسلي.

العينات الموجبة للتزريع خضعت للاختبارات البيوكيميائية و أظهرت (100)

لحامض البرانايتروبنزويك (تم تثبيط النمو بواسطة حامض البرانايتروبنزويك) مقاومة للتوفين 2- حمض كاربوكسيليك اسيد هيدرازيد ، موجبة لاختبار اختزال النترات وكانت سلبية لاختبار الكتاليزفي درجة حرارة 68 °م.

اظهرت اختبارات الحساسية للادوية ان 26 (20.3) منقطرة ، متعددة المقاومة للادوية 16 (34.8) ، للنوع الاول من الادوية، سلالتين (4.4) مقاومة فقط للستربتوميسين ، وسلالتين ثلاثية المقاومة للايرونيزايد، الستربتوميسين و الإيتامبوتول. ستة واربعون (36) من عزل المنقطرة السلي اختبرت بواسطة تفاعل البلمرة التسلسلي. (100) من العزلات اظهرت حزمه مطابفه في القياس (123 bp) للجين المستهدف (IS 6110). ستة وعشرون من عزلات المنقطرة السلي المقاومة للادوية اختبرت بواسطة تفاعل البلمرة التسلسلي للجين المقاوم للرفامبسرين اظهرت حزمه مطابفه في القياس (193bp) (20.3) ، كانت من بينهم (86.7%) مقاومة للجين (rpoB). اما فيما يخص المجموعة ولاية الجزيرة خمسة وعشرون (25%) من عزلات المنقطرة السلي المقاومة للادوية اختبرت بواسطة تفاعل البلمرة التسلسلي للجين المقاوم للرفامبسرين وكانت موجبة، بينما تسعة عشر (19%) اظهرت حزمة مطابقة في القياس (193bp) (19) كانت من بينهم (76%) مقاومة للجين (rpoB).

نتيجة لانخفاض حساسية الصبغه المقاومة للاحماض الفترة الطويلة المستغرقة لاداء التزريع واختبار الحساسية للادوية اظهرت النتائج بوضوح اهمية وحساسية وجدوى تفاعل البلمرة التسلسلي كاداة سريعة للتشخيص والكشف عن المنقطرة السلبية وللجين المقاوم للرفامبسرين. كما انه يمكن استخدام تفاعل البلمرة التسلسلي مباشرة من الحشعة المريض. اوصت الدراسة بضرورة اعتبار تفاعل البلمرة التسلسلي كاداة تشخيصية لمرض السل، كما اوصت بدعم وتطوير وسائل التشخيص الحديثة وتقييم نتائجها السريعة والدقيقة.

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