بسم الله الرحمن الرحيم

قال تعالى :

وَقُلْ رَبِّ زِدْنِي عِلْمًا}

صدق الله العظيم

سورة طه الآية (114)

Dedication

I dedicate this research to

My father.....

My mother....

My brothers and sisters....

For their support and kindness

My friends and my colleagues.....

Acknowledgment

First of all my thanks to Allah for giving me the ability and strength to accomplish this work

I'm deeply indebted to my supervisor Dr.Mogahid Mohamed Elhassan, Head of Microbiology Department, Sudan University of Science and Technology, for facilitating the completion of my research by providing support, advice and encouragement through the course of the study.

Thanks are also extended to the member of the Tuberculosis Reference Laboratory, National

Health Laboratory, especially Miss. Eman Osman, Mr. Ahmed Hassan

Special thanks love go to my parents, sisters and brothers specially Roaa for their support throughout the duration of the study.

Abstract

The aim of this study was to isolate Mycobacterium other than Tuberculosis (MOTTs) among patients with pulmonary infection in Khartoum State and to evaluate the usefulness of polymerase chain reaction (PCR) as a rapid test that may substitute the former tedious technique.

171 sputum samples were collected from suspected pulmonary tuberculosis patients at the National Health Laboratory, Elasha'ab Teaching Hospital, Abo Anga Hospital, Ibrahem Malik Hospital and Academy Charity Hospital. Patients were consented and informed.

All sputum samples (171) were inculcated. 40 (23.4%) of isolates give characteristic growth of *Mcobacterium tuberculosis*, were identified according to their Ziehl-Neelsen stain, and cultural characteristic. 10 (9%) isolate identify as rapidly grower 6 out of 10 isolates were identified as MOTTs according to their indirect Ziehl-Neelsen stain, cultural characteristic and biochemical proprieties.

Ten rapidly grower isolates were subjected to PCR. Six isolate showed a band typical in size (136 bp) to target gene (*rpoB*) as indicated by the standard DNA marker. Two isolates were showed negative result for the target gene.

In this study, males were found to be more affected than females (73.3%). The patient between 21-50 years was the most affected with TB and NTM.

The results obtained from this study revealed clearly the importance of conventional methods including Z.N stain and culture techniques in the diagnosis of TB and NTM, and the effectiveness of PCR as rapid and promising diagnostic tool which may substitute the formal tedious technique.

الخلاصة

الهدف من هذه الدراسة هو عزل المتفطرة غير السلية بين المرضي الذين يعانون من التهاب رئوي ، وتقييم مدى فائدة اختبار البلمرة التسلسلي كاختبار مؤكد.

جمعت 171 عينة قشع من مرضي يشتبه في اصابتهم بلدرن الرئوي من المعمل القومي الصحي, مستشفي الشعب التعليمي, مستشفي ابوعنجة, ومستشفى الأكاديمية الخيري في ولاية الخرطوم. المرضى تم إعلامهم بهذه الدراسة.

كل عينات القشع تم تزريعها. (23.4%) 40 من العينات المعزولة أظهرت مظهر عضيات الباكتيريا المتفطرة الدرنية, تم التعرف عليه اعتمادا علي خصائصها المجهرية و المزرعية. 10 (5.8%) عزلات تم التعرف عليها كمتفطرة سريعة النمو. 6 من اصل 10 عزلات تم التعرف عيها كمتفطرة غير سلية اعتمادا علي خصائصها المجهرية غير المباشرة, المزرعية و الاختبارات البيوكيميائية.

عشر عزلات من المتفطرة سريعة النمو, تم إختبارها بواسطة تفاعل البلمرة التسلسلي. ستة عزلات أظهرت حزمة مطابقة في القياس للجين المستهدف (*rpoB*) كما هو مشار إليه بواسطة المؤشر القياسي للحامض النووي الرايبوزي منزوع الأوكسجين. بينما أظهرت عزلاتان نتائج سالبة.

في هذه الدراسة ، وجد أن الرجال كانوا أكثر تضرر من الإناث (73.3٪). الفئة العمرية 50 21- كانت الأكثر تأثرا بالمتفطرة السلية والمتفطرة غير السلية.

النتائج التي تم الحصول عليها من هذه الدراسة كشفت بوضوح اهمية الأساليب التقليدية التي تشمل تقنيات صبغة زيهل نيلسن والتزريع في تشخيص المتفطرة السلية والغير سلية, ايضا فعالية تفاعل البلمرة التسلسلي كاختبار تشخيصي سريع وواعد قد يحل محل التقنية السابقة المملة.

List of Contents

الآية	I
Dedication	II
Acknowledgment	III
Abstract	IV
Abstract (Arabic)	V
Table of Contents	VI
List of Figures	X
1. CHAPTER ONE: Introduction and Objectives	1
1.1 Introduction	1
1.2 Rationale	4
1.3 Objectives	5
2. Chapter TWO: LITERATURE REVIEW	6
2.1 *Mycobacterium*	6
2.1.1 Definition and Taxonomy	6
2.1.2 Clinical Importance of *Mycobacterium* Species	7
2.1.2.1. *Mycobacterium tuberculosis*	7
2.1.2.2. Non-Tuberculous *Mycobacteria*	7
2.2. History	8
2.3. Classification according to Timpe and Runyon	9
2.3.1.Type I: Photochromogens (Slowly growing)	9
2.3.1.1 Mycobacterium kansasii	9
2.3.1.2 Mycobacterium marinum	10
2.3.1.3 Mycobacterium simiae	10
2.3.2 Type II: Scotochromogens (Slowly growing)	11
2.3.2.1 Mycobacterium scrofulaceum	11
2.3.2.2. Mycobacterium gordonae	11
2.3.3.Type III: Nonchromogenic (Slowly growing)	12
2.3.3.1. Mycobacterium avium complex (MAC)	12
2.3.3.2. Mycobacterium xenopi	12
2.3.3.3 Mycobacterium terrae Complex	13
2.3.4 Type IV: Rapid Growers	13
2.3.4.1 Mycobacterium fortuitum Complex	13
2.4 Epidemiology	14
2.5 Pathogenesis	15
2.5.1 Pulmonary Disease	17

2.5.2 Lymphatic Infection	18
2.5.3. Skin and Soft Tissue Infection	19
2.5.4. Infection of Bones and Joints	20
2.5.5 Disseminated Disease	20
2.6 Laboratory Diagnosis	21
2.6.1 Skin Test	21
2.6.2 Specimens	21
2.6.21Respiratory Specimens	22
2.6.2.2 Specimen Processing	22
2.6.4 Traditional Culture Techniques	23
2.6.41 Broth Media	23
2.6.4.2 Solid Media	24
2.6.4.3 Incubation of NTM Cultures	25
2.6.5. Biochemical Tests and Morphological Features	25
2.6.5.1 Niacin accumulation test	26
2.6.5.2 Nitrate Reduction Test	26
2.6.5.3 Catalase Test	26
2.6.6 Nucleic Acid Technology	27
2.7 Treatment and Control	27
CHAPTER Three : Materials and method	28
3.1 Study Design	28
3.1.1. Type of the Study	28
3.1.2. Study Area	28
3.1.3 Study Population	
3.1.4 Inclusion Criteria	28
3.2 Asepsis and Sterilization	28
3.2.1 Flaming	28
3.2.2 Red Heat	29
3.2.3 Hot air Oven	29
3.2.4 Moist Heat (autoclaving)	29
3.2.5 Disinfection	29
3.3 Collection of Specimens	29
3.4. Staining (Ziehl- Neelsen’s stain)	29
3.5. Decontamination of Sputum	30
3.6 Preparation of Culture Media	30
3.6.1 Preparation of Lowenstein Jensen Medium with Glycerol	31
3.6.1.1 Preparation of Mineral Salts	31
3.6.1.2 Malachite Green Solution 2%	31

3.6.1.3 Preparation of homogenized whole eggs	31
3.6.1.4 Preparation of complete medium	31
3.7 Culture Method	32
3.8 Identification of Isolate	32
3.8.1 Growth Rate	32
3.8.2 Pigment Production	33
3.9 Biochemical Testing	33
3.9.1 Catalase Test	33
3.9.2. Nitrate Reduction Test	34
3.9.3. Sensitivity to Para – Nitrobenzoic Acid (PNB) 500 mg / L	34
3.9.4. Sensitivity to Thiophene -2- Carboxylic Acid Hydrozide (TCH) 5 mg / L	34
3.10. Molecular Identification by Using Polymerase Chain Reaction (PCR)	34
3.10.1. DNA Extraction from Cultures	35
3.10.2. Design of the Primers	35
3.10.3. Preparation of PCR Mixture	35
3.10.4. PCR Amplification	36
3.10.5. Preparation of Agarose Gel	36
3.10.6. Visualization of PCR Product	36
Chapter Four: Result	**38**
4.1. Epidemiological finding	38
4.1.1 Gender	38
4.1.2 Age groups	39
4.2 Bacteriological Finding	40
4.2.1 Direct Ziehl-Neelsen Smear	40
4.2.2 Growth Rate	40
4.2.3 Colonial Morphology	41
4.2.4 Indirect Ziehl-Neelsen Smear	42
4.2.5 Biochemical tests	42
4.3 Polymerase chain reaction	44
Chapter five: Discussion	46
Chapter Six	48
Chapter Six Conclusion and Recommendations	48
6.1 Conclusion	48
6.2 Recommendation	49
REFERENCES	50
APPENDIX I Z.N. staining	25
APPENDIX II Decontamination Of Sputum	53

APPENDIX III catalase Test	53
APPENDIX IV Nitrate Reduction Test	55
APPENDIX V Sensitivity To Para – Nitrobenzoic Acid (PNB) 500 mg /	55
APPENDIX VI Sensitivity To Thiophene - 2 – Carboxylic Acid Hydrozide (TCH) 5 mg / l	56
APPENDIX VII PCR Reagents	56
APPENDIX VIII PCR equipments	57
APPENDIX IX Table 1 Result of Biochemical Tests	58

List of Figure

Figur1. Distribution of patients with pulmonary infection according to gender	38
Fig2. Distribution of MTB and NTM according to age group.	39
Plate 3. Showing characteristic growth of NTM in LJ medium.	41
Plat 4. showing results of Catalase test; positive to the left side and negative on right side	42
Plat 5. showing results of nitrate reduction test from the left; first tube is negative, 2= is faint, 3= +, 4= ++, 5= +++, 6= ++++	43
Figure6. The amplicon of NTM after first run of PCR on 1% Agarose gel	44
Figure7. The amplicon of NTM after first run of PCR on 1% Agarose gel	44
Figure8. The amplicon of NTM after first run of PCR on 1% Agarose gel	45