

STUDY ON THE INCREASING CLINICAL IMPORTANCE OF THE ATYPICAL MYCOBACTERIA " TUBERCULOID BACILLI " WITH SPECIAL EMPHASIS ON ITS LABORATORY DIAGNOSIS AND TREATMENT

Essay

Submitted for Partial Fulfillment of Master Degree in Clinical and Chemical Pathology

By

Dr. Seham Abd El-Hamed Radwan Hasan
M.B.B.ch. 6398 1

616.07 S. A

Supervisors

Prof. Dr. Ragaa Mahmoud Lasheen

Prof. of Clinical and Chemical Pathology
Ain Shams University

Dr. Amira Mohamed Mokhtar

Assistant Prof. of Clinical and Chemical Pathology Ain Shams University

Faculty of Medicine Ain Shams University 1995



ACKNOWLEDGEMENT

I would like to express my special thanking and appreciation to professor **Dr. Ragaa Mahmoud Lasheen** professor of clinical and chemical pathology, Faculty of Medicine - Ain shams University for her motherly encouragement, valuable guidance, and sincere help.

I wish to extend my sincere gratitude to **Dr. Amira**Mohamed Mokhtar Assistant professor of clinical and chemical pathology, Faculty of Medicine -Ain Shams university for her kind support and supervision.

I also express my sincerest thanks to **Dr. Nahed Afify** lecturer of clinical and chemical pathology, Faculty of Medicine -Ain shams University who offered this study much of her time and smart concepts.

I would also like to thank my husband and my family for being very supportive and understanding.

CONTENTS

		Page ?			
-	INTRODUCTION AND AIM OF THE WORK	1			
_	REVIEW OF LITERATURE				
I.	Tuberculoid Bacilii				
	- Historical aspect	5			
	- Classification	7			
	- Morphology and structure of the cell wall	11			
	- Environmental and growth requirement	13			
	- Antigenicity	16			
	- Immune response	17			
	- Epidemiology	19			
	- Resistance to physical and chemical agents	21			
IJ.	Tuberculoid Bacilli Infections	23			
	- Pathogenesis	26			
	- Clinical significance	28			
	- Clinical Manifestations.	29			
	- Radiological findings.	30			
	- Differential diagnosis.	31			
	- Prognosis.	31			
	- Other Organisms Less Commonly	32			
	Causing Pulmonary Disease Extrapulmonary Diseases Caused by Tuberculoid Bacilli:	33			
	- Tuberculoid bacilli infection in patients with AIDS	36			
III	Laboratory diagnosis of tuberculoid bacilli infection	40			

-	Specimen collection and processing	40
-	Direct methods of diagnosis	46
	- Staining and microscopy.	47
	- Culture and environmental requirement	52
	- Identification of isolates	60
	- Rapid identification:	60
	 Identification by radioactive methods (BACTEC system) 	60
	- Biphasic Septi-Chek culture media	62
	- Identification by molecular method	64
	- Traditional approach :	66
	- Presumptive identification:	66
	 Rate of growth and growth in relation to temperature 	66
	- Pigmentation and photoreactivity.	68
	- Definitive identification	68
	- Chromatography	78
	- Direct specimen assay	81
	- Antimicrobial susceptibility	85
-	Drug resistance	88
-	Indirect methods of diagnosis	90
-	Skin test.	91
ſV	Treatment of tuberculoid bacilli infection	95
V	Prevention of tuberculoid bacilli infection	100
	SUMMARY	102
	REFERENCES	106
	ADARIC SUMMADV	

LIST OF ABBREVIATIONS

AE-DNA : Acridinium ester-labelled-DNA

AFB : Acid fast bacilli

AIDS : Acquired immunodeficiency syndrome

BAL : Bronchoalveolar lavage
BCG : Bacille calmette Guerin
CFU : Colony-forming unit

CIP : Ciprofloxacin CLA : Clarithromycin

CPC : Celylpyridinium chloride CSF : Cerebrospinal fluid

ELISA : Enzyme linked immunosorbent assay

GI : Growth index

GLC : Gas-liquid chromatography
HIV : Human immunodeficiency virus
HPA : Hybridization protection assay

HPLC : High performance liquid chromatography

IFN : Interferon

L-J : Lowenstein-Jensen M. : Mycobacterium

MAC : Mycobacterium avium complex
MAI : Mycobacterium avium-intracellulare

MAIS : Mycobacterium avium-intracellulare-schrofulaceum

MB : Middlebrook

MOTT : Mycobacterium other than tubecle bacilli

M.TB : Mycobacterium tuberculosis

NALC: N-acetyl-L-cysteine

NAP p-nitro-α-acetylamino-β-hydroxypropiophene

PBL : Prepheral blood lymphocyte
PCR : Polymerase chain reaction
PPD : Purified protein drivative
Py-MS : Pyrolysis mass spectrometry

RIF : Rifambin T.B. : Tuberculosis

TCH Thiophene-2-carboxylic acid hydrazide

TLC: Thin-layer chromatography
TSA: Tuberculostearic acid

List of Tables

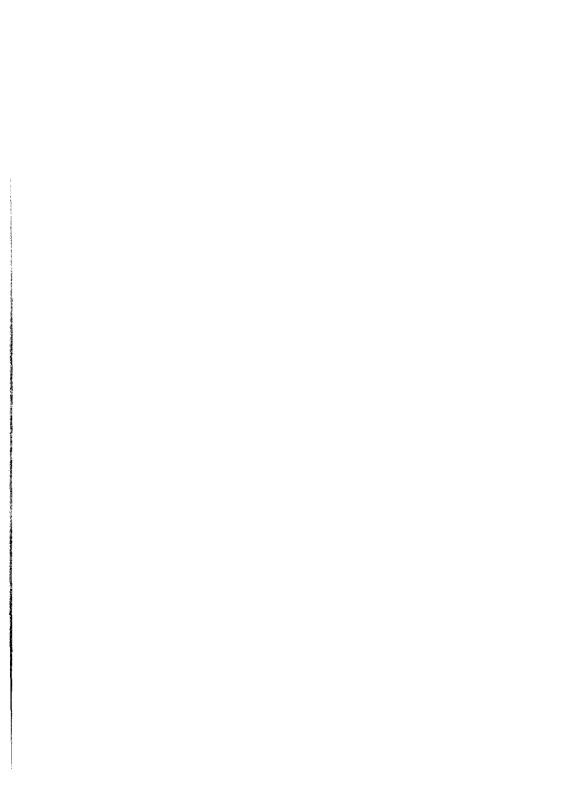
			Page
Table, I	:	Recognized Species of Mycobacteria	8
Table, П	:	Growth of Mycobacteria.	15
Table, III	:	Clinical Significance of Atypical	24
		Mycobacteria.	
Table, IV	:	Reporting Acid-Fast Bacilli in Fuchsin	52
		Stained Smears.	
Table, V	:	Non-selective Mycobacterial Solid Isolation	54
		Media.	
Table, VI	:	Selective Mycobacterial Isolation Media.	56
Table, VII	:	Morphologic Characteristics of Clinically	58
		Significant Mycobacteria.	
Table, VIII	:	Distinctive Properties of Mycobacteria	70
		Encountered in Clinical Specimens	

List of Figures

		Page
Figure, I	: A flow Chart for Specimen Processing for	44
	Isolation of Mycobacteria.	
Figure, II	: Algorithm for Processing BACTEC 12B	63
	Bottles for Probe.	
Figure, III	: Preliminary Subdivision of Mycobacteria.	67
Figure, IV	: The Identification of Mycobacteria.	78



INTRODUCTION AND AIM OF THE WORK



INTRODUCTION

Genus Mycobacterium is one of the most widely distributed bacterial genera in nature. The generic name Mycobacterium was given to a group of bacteria which grow as mould-like pellicles on liquid media (Grange, 1994). Genus Mycobacterium comprises, a large group of acid-fast, alcoholfast, aerobic or microaerophilic, non-motile, non-sporforming bacilli, 0.2 to 0.6 x 1 to 10 µm in size. They occasionally form branched filaments, but these can be readily disrupted (Murray et al., 1994).

The lipid content of mycobacterial cell wall is very high, the most characteristic components are genus-specific, alphahydroxy branched chain fatty acids of high molecular weight "mycolic acid". The mycobacterial cells are difficult to stain, but once stained resist decolourization with acid and alcohol. They are Gram-positive, but some species are poorly colored even after prolonged staining. They are straight or slightly curved rods, but coccobacillary, filamentous and branched forms also may occur (Laidlaw, 1989).

This bacteria have a generation time of approximately 20 hours, and thus their isolation and identification may take up to 6 weeks. Catalase, niacin production, reduction of nitrate to nitrite and many other tests used to diagnose mycobacterial species (Hall and Howard, 1994). The genus Mycobacterium includes numerous pathogens and saprophytic organisms. They includes over 30 species, most of which are well defined. The commoner species are classified into:

- 1. The typical tubercle bacilli, *M.tuberculosis* and *M.bovis*.
- 2. The atypical mycobacteria, including commensal,

saprophytic, and opportunistically pathogenic species.

- 3. The Mycobacterium leprae.
- 4. The strict animal pathogens (Baron et al., 1994).

The atypical mycobacteria have many other names, including pseudotubercle bacilli, unclassified mycobacteria, non-tuberculous mycobacteria, tuberculoid bacilli, opportunistic mycobacteria, environmental mycobacteria, anonymous mycobacteria, and mycobacteria other than tubercle bacilli "MOTT" which is the preferred term (Yeager and Jr, 1994). Many tuberculoid bacilli occasionally cause opportunistic infections in man indistinguishable clinically, radiologically and histologically from that caused by the human tubercle bacilli (Lillo et al., 1990). And may present diagnostic and therapeutic difficulties (Hopkin, 1995).

The rate of isolation of MOTT had increased over the past several years; in some areas, the isolation rate for Mycobacterium avium- intracellulare has risen than that for Simultaneously, the spectrum of clinical M.tuberculosis. manifestations associated with the various species has widened. Mycobacteria other than tubercle bacilli differ from M.tuberculosis in several respects. They are widely spread in nature, and their pathogenic potential for humans varies; they may colonize on individual without causing invasive disease (Hoover, 1995). Thus in contrast to M.tuberculosis which is always considered a pathogen when isolated, but MOTT, when isolated are not necessarily equated with disease (Shafer and Sierra, 1992). The isolation of a tuberculoid bacillus on a single occasion is not a sufficient evidence that it is the cause of the patient's illness; it may be a secondary invader or a contaminant. Three to six isolations of the organism are