EVALUATION OF DRUG SUSCEPTIBILITY PROFILE AMONG CATEGORY II PULMONARY TUBERCULOSIS PATIENTS (RELAPSE, FAILURE, DEFAULTER) AT TIRUNELVELI

DISSERTATION SUBMITTED FOR

M.D. (BRANCH IV)

MICROBIOLOGY

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY,

CHENNAI, TAMILNADU

MARCH – 2009

CERTIFICATE

This is to certify that the dissertation entitled, ‘‘Evaluation of Drug Susceptibility Profile among Category II Pulmonary Tuberculosis patients (Relapse, Failure, Defaulter) at Tirunelveli” by Dr.V.P.Amudha, Post graduate in Microbiology (2006-2009), is a bonafide research work carried out under our direct supervision and guidance and is submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai, for M.D. Degree Examination in Microbiology, Branch IV, to be held in March 2009.

GUIDE: **(Dr. N. Palaniappan)**
Professor and Head,
Department of Microbiology,
Tirunelveli Medical College,
Tirunelveli –11.

CO GUIDE: **(Dr. Ranjani Ramachandran)**
Scientist D,
Tuberculosis Research Centre,
Chetput, Chennai - 600031.

CO GUIDE: **(Dr. R. Ramnath)**
Tutor,
Department of Thoracic Medicine
Tirunelveli Medical College,
Tirunelveli –11.

Date:

Place:

ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of Tirunelveli Medical College and hospital has approved the study ,

"EVALUVATION OF DRUG SUSCEPTIBILITY PROFILE AMONG CATEGORY II PULMONARY TUBERCULOSIS PATIENTS (RELAPSE, FAILURE, DEFAULTER) AT TIRUNELVELI"

submitted by Dr.V.P. Amudha, Post Graduate in Microbiology, Tirunelveli Medical College, following the regulations and guidelines.

Date:

Place:

CHAIRMAN

FOR ETHICAL COMMITTTEE

Office seal:

Acknowledgement

ACKNOWLEDGEMENT

I express my heartfelt gratitude to the Dean, Tirunelveli Medical College, Tirunelveli, for all the facilities provided for the study.

I honestly thank my guide Dr. N. Palaniappan, M.D., Professor and Head, Department of Microbiology, Tirunelveli Medical College, for his support, guidance, criticism, advice and constant encouragement all through this investigation.

I am greatly indebted to **Dr. Ranjani Ramachandran**, Scientist D, Tuberculosis Research Centre, Chennai, for her expert guidance in midst of her workload all through this work.

I am highly thankful to **Dr. R. Ramnath**, Tutor, Department of Thoracic Medicine, Tirunelveli Medical College, for his guidance, fervent advice, motivation and constant support while carrying out this work.

I am grateful to **Dr. S. Poongodi @ Lakshmi**, M.D., Reader, Department of Microbiology, Tirunelveli Medical College, for her sincerity in critically reading this work and providing valuable and constructive comments on my work.

I am much beholden to Dr. K. Muthulakshmi, M.D., Department of Microbiology, Tirunelveli Medical College Hospital, for her motivation to perform this work.

I am very grateful to Dr. C. Revathy, M.D., Reader, Department of Microbiology, Tirunelveli Medical College, for the encouragement in every stage of this work.

I am highly obliged to Dr. V. Sadiqua, M.D., Dr. P. Sornajeyanthi, M.D., Dr. T. Jeyamurugan, M.D., Assistant Professors and Dr. M. A. Ashika Begum, M.D., Dr. B. Cinthujah, M.D., Tutors Dr. G. Velvizhi, M.D., Dr. G. Sucila Thangam, M. D., Department of Microbiology, Tirunelveli Medical College, for being with me to support all my endeavours throughout the study.

I gratefully acknowledge the receipt of financial assistance from The State Health Society RNTCP for pursuing this research work.

Special thanks are due to my co-postgraduate colleague Dr. S. Nithya Gometheswari and Dr. S.

Anandha Sankari for their technical help and peerless support.

Thanks are due to the, Messer. N. Jeyaraman, V. Parthasarathy,V. S. Bhuhari, Selvakumar, U. Chandran, K. Umaivel, Tmt.Malarvizhi, Tmt.Premalatha and Tmt.Shanthi, supporting staffs for the services rendered.

I am highly obliged to Dr. K.Krishnamurthy, Dept of Thoracic medicine, for his help and suggestions to carry out the work. Special thanks to Mr. B Thirumalaikumar, Mr. T Kathirvelmurugan, Lab technicians and all the staff of Dept of Thoracic Medicine for their kind help in specimen collection.

I owe an immeasurable debt of thanks to Mrs. Nalini Sundarmohan, Sr.Technical officer, TRC, for all the help she rendered me at the centre. I deeply mourn her sudden demise in an accident on 8-11-2008.

I owe my thanks to Miss. R. Laksmi, JRF, TRC, for her immense help in analyzing the data and preparing the manuscript. I thank all the staff of TRC for their unstinted technical guidance throughout the research period.

I thank **Mr. Arumugam** who helped me in the statistical analysis of the data.

And, of course, I'm most indebted to my Beloved family, my friends and THE ALMIGHTY.

Contents

CONTENTS

Introduction

1. INTRODUCTION

Tuberculosis is a disease of great antiquity. There is evidence of spinal tuberculosis in Neolithic, pre Columbian and early Egyptian remains. It was described in the Vedas and other Hindu texts as Rajyakshma-the king of diseases. Tuberculosis became a major problem during the industrial revolution, when crowded living conditions favoured its spread. Though the disease was known since ancient times, the organism causing tuberculosis was described only a century ago by Robert Koch on 24th March 1882.

1.1 The agent

The term tubercle bacillus designates two species of the family Mycobacteriaceae, order Actinomycetales: *M. tuberculosis and M. bovis*. Three other species – *Mycobacterium microti*, a pathogen for rodents, *Mycobacterium africanum* and *Mycobacterium canetti*, both rare causes of tuberculosis in Africa are closely related and are the other members of the M. tuberculosis complex. The complex also contains BCG vaccine, derived from a strain of *M.bovis*. Disease caused by M. bovis is relatively rare, and the terms tubercle bacillus and Mycobacterium tuberculosis are synonymous.

1.2 The genome

Evenly distributed genes along both the strands of DNA of *M.tuberculosis* and single copy of the ribosomal RNA operon located at unusually 1500 kb from the origin of replication explains the slow growth and its 24 hr generation time. Presence of genes encoding aerobic metabolism and anaerobic electron transport chain enable the bacilli to survive in granulomas or oxygen poor tissues. The high genetic variability of PE and PPE families of glycerin rich proteins interfere with host immune responses by inhibiting antigen processing. The richness of insertion sequences (IS) may be the source of chromosomal rearrangements and deletions *(Topley and Wilsons p.1186).*

1.3 The Pathogenesis

Airborne droplet nuclei containing tubercle bacilli reach the terminal air spaces where

multiplication begins. The bacteria are ingested by alveolar macrophages, which may be able to eliminate small numbers of bacilli. Protective immunity is mainly cell mediated. However, the bacterial multiplication tends to be mostly unimpeded and can survive inside the macrophages by preventing the oxidative burst and phagosome lysosome fusion. The bacilli resist lysosomal enzymes and ROI by virtue of cell wall lipids. Multiplication of bacilli leads to cell death and local inflammation which attract more phagocytes to the site. Some are transported to the regional lymph nodes and others further afield causing extra pulmonary tuberculosis.

Epitopes from mycobacteria are presented by the APCs to CD4+ cells, which on activation produce a range of cytokines including IFN-γ that activate the macrophages to form the granulomas and multinucleated giant cells. Cytotoxic CD8+ T cells are also generated to lyse the infected mononuclear phagocytes directly. The center of granuloma is anoxic and caseous necrosis occurs which kills many bacilli. TNF-α plays a key role in protective immunity by maintaining the integrity of the granuloma. In about 95% of the primarily infected patients these defense mechanisms render the disease quiescent, but some may survive for years or decades in a latent state to be reactivated later *(Topley and Wilsons p.1202).*

1.4 Revised National Tuberculosis Control Programme Guidelines

RNTCP is an application in India of the WHO recommended Directly Observed Treatment Short Course (DOTS) strategy to control Tuberculosis. Tuberculosis cases are classified as **pulmonary and extra-pulmonary**.

1.4.1 Pulmonary tuberculosis

Pulmonary Tuberculosis is further classified as smear positive and smear negative.

a. Smear-positive patient

- 2 sputum positive for acid-fast bacilli (AFB).

- 1 sputum positive for AFB + Radiographic abnormalities.

- 1 sputum positive for AFB + Culture positive for *M.tuberculosis*.

b. Smear-negative patient

- 3 sputum negative for AFB but with Radiographic abnormalities.

- 3 sputum negative for AFB but Culture positive for *M. tuberculosis.*

Seriously ill smear-negative Pulmonary TB cases are

-Miliary Tuberculosis, Extensive parenchymal infiltration, Co-infection with HIV, all forms of pediatric sputum smear negative pulmonary tuberculosis except primary complex.

1.4.2 Extra Pulmonary Tuberculosis (EPTB)

This includes Tuberculosis of organs other than the lungs. Patients with both pulmonary and extra-pulmonary are classified as having Pulmonary Tuberculosis.

Seriously ill EPTB cases are described in table 1.1.

Any patient, with Pulmonary or Extra Pulmonary Tuberculosis, who is **HIV positive,** is considered as seriously ill**.**

1.4.3 Case definitions

Tuberculosis patients are designated as

New - never had treatment for Tuberculosis or had treatment for less than a month.

Relapse - declared cured or treatment completed, but found to be sputum positive later.

Treatment after default - had ATT for one month or more and returns to treatment after not taking drugs consecutively for two months or more, and is sputum positive.

Failure - smear positive after 5 months of treatment and Category III patients who becomes smear positive during treatment.

Chronic - remains smear positive after completing re-treatment regimen.

The categories of treatment and the schedule for follow-up sputum examinations during entire course of treatment under RNTCP are described in table 1.2 and 1.3 respectively.

1.5 Drug resistance

As the programme strives hard to achieve its goals, it is at present facing the threat of drug resistance, a menace that would destabilize the tuberculosis control.

Drug resistance as a limiting factor for success of chemotherapy was recognized immediately following the introduction of Streptomycin. *Youmans et al 1946* found that when Streptomycin was given alone, there was a rapid decrease in the number of bacilli in the sputum which however increased again. *Pyle (*1947) showed that during treatment with Streptomycin alone, the proportion of drug-resistant bacilli increased progressively from about 1 in 88,750 organisms before therapy, to about 1 in 367 after 15 weeks of treatment. Studies by *Crofton and Mitchison (1948)* showed that with monotherapy or inadequate therapy, the number of susceptible bacilli **Table**

1.1 Extra Pulmonary Tuberculosis (EPTB)

Seriously ill EPTB	Not seriously ill EPTB
• Meningitis • Pericarditis • Peritonitis • Bilateral or extensive pleural effusion • Spinal TB with neurological involvement • Intestinal • Genito-urinary • Co-infection with HIV • All forms of pediatric Extra pulmonary tuberculosis other than lymph node tuberculosis and unilateral pleural effusion.	• Lymph node • Pleural effusion (unilateral) • Peripheral joints

Table 1.2 Categories of Treatment

Category of Treatment	Type of Patient	Regimen*	
		Intensive phase	Continuation phase
Category I	New sputum smear-positive Seriously ill new sputum smear-negative Seriously ill new extra-pulmonary	$2H_3R_3Z_3E_3$	$4H_3R_3$
Category II	Sputum smear-positive Relapse Sputum smear-positive Failure Sputum smear-positive Treatment After Default	$2H_3R_3Z_3E_3S_3$ + $1H_3R_3Z_3E_3$	$5H_3R_3E_3$
Category III	New Sputum smear-negative, not seriously ill New Extra-pulmonary, not seriously ill	$2H_3R_3Z_3$	$4H_3R_3$

**The number before the letters refers to the number of months of treatment. The subscript after the letters refers to the number of doses per week. The dosage strengths are as follows: H: Isoniazid (600 mg), R: Rifampicin (450 mg), Z: Pyrazinamide (1500 mg), E: Ethambutol (1200 mg), S: Streptomycin (750 mg).*

Table 1.3 Schedule for follow-up sputum examinations during entire Course of treatment

Category	Pretreatment sputum	Test at month	If result is	Then…
Category I	Positive	2	NEG	Start continuation phase, test sputum again at 4 and 6 months
			POS	Continue intensive phase for one more month, test sputum again at 3, 5 and 7 months
	Negative	2	NEG	Start continuation phase, test sputum again at 6 months
			POS	Continue intensive phase for one more month, test sputum again at 3,5 and 7 months
Category II	Positive	2	NEG	Start continuation phase, test sputum again at 5 and 8 months
			POS	Continue intensive phase for one more month, test sputum again at 4,6 and 9 months
Category III	Negative	2	NEG	Start continuation phase, test sputum again at 6 months
			POS	Re-register the patient and begin Category II treatment

(*RNTCP Training Module, 2006)*

decreased, while the resistant bacilli increased in lung cavities of the patients. This was called the **‘‘fall and rise”** phenomenon.

Temple and associates (1951) showed that multiple drug therapy would prevent development of drug resistance and tuberculosis patients were then treated only with multiple drugs.

There are several antimicrobial drugs currently being used for the treatment of Tuberculosis. These include first line drugs such as Isoniazid, Rifampicin, Streptomycin, Pyrazinamide and second line drugs like Aminoglycosides (Amikacin, Kanamycin), Quinolones, Para Amino Salicylic acid, Cycloserine, Thioamides (Ethionamide, Prothionamide), Polypeptides (Capreomycin).

1.5.1 WHO Definitions of Drug Resistance

Drug resistance among new cases:

- the presence of resistant isolates of *M.tuberculosis* in patients who, in response to direct questioning, deny having had any prior anti-TB treatment (for as much as 1 month).

Drug resistance among previously treated cases:

- the presence of resistant isolates of *M.tuberculosis* in patients who, in response to direct questioning, admit having been treated for tuberculosis for 1 month or more *(Anti-tuberculosis drug resistance in the world, WHO Fourth Global Report 2008).*

Multi-Drug Resistant Tuberculosis (MDR-TB):

Resistance to Isoniazid and Rifampicin with or without resistance to other first line drugs.

Extensively Drug-Resistant Tuberculosis (XDR TB):

MDR-TB with added resistance to at least two (Flouroquinolones & Injectable agent) of the six main classes of second line drugs.

1.5.2 Mechanism of Resistance in Mycobacterium Tuberculosis

Genetic mutation:

Resistance in *M.tuberculosis* is always a result of mutations confined to chromosomal DNA.

The MDR phenotype is caused by accumulation of mutations involved in individual drug resistance. The probability of resistance is

Very high: - Thiacetazone, Ethionamide, Capreomycin and Cycloserine (10^{-3}); Intermediate: - INH, SM, EMB, Kanamycin, and PAS (10^{-6});

Lowest: - Rifampicin (10^{-8}). *Shimao 1987; Crofton 1970).*

The mutation rate is directly proportional to the bacterial load. These mutations being chromosomal, simultaneous resistance to two or more drugs is extremely unlikely. Serial selection of drug resistance is the predominant mechanism for the development of MDR strains.

Mutants resistant to one drug are, as a rule, susceptible to other and vice versa. Only mutants resistant to both drugs simultaneously are a cause of concern when the concentration is exceptionally low (C*anetti 1961)*, which is rare. When bacterial population diminishes, there is little chance for mutants resistant to one drug and no likelihood of presence of multi resistant mutants. The various Gene Loci conferring drug resistance to MTB is listed in Table -1.4.

Table 1.4 Various Gene Loci conferring drug resistance to MTB

Drug	**Gene**	**Gene product/functional role**	**Cellular target**
RIF	***rpoB***	β-subunit of RNA polymerase/transcription	Nucleic acids
INH	***katG***	Catalase-peroxidase/activation of Pro-drug	Cell Wall
	inhA	enoyl-acyl carrier protein reductase / mycolic acid biosynthesis	
	oxyR-ahpC	Alkyl-hydro-reductase/unknown	

	kasA	b-ketoacyl acyl carrier protein/ mycolic acid biosynthesis	
SM	***rspl*** ***rrs***	Ribosomal protein S12/translation 16S rRNA/translation	Protein synthesis
FQ	***gyrA***	DNA gyrase subunit / DNA replication	Nucleic acid
PZA	***pncA***	pyrazinamidase-nicotinamidase / activation of prodrug	Unknown
EMB	***embB***	Arabinosyl transferase/arabinan polymerization	Cell Wall

Role of multidrug transporters:

Low-level drug resistance is due to multidrug efflux systems of bacterial cells which limit the access of antimicrobial agents to their targets.

P-glycoprotein is a human analogue of these multidrug transporters and is expressed on immune effector cells *(Verbon et al 2002).* It has been observed that infection of experimental cell lines by *M.*

tuberculosis results in increased expression of P-glycoprotein and decreased accumulation of Isoniazid inside the cells *(Gollapudi et al 1994).* Apart from the up regulation of host cell P-glycoprotein, *M.tuberculosis per se* expresses at least three multidrug transporter proteins Tap, Lfr *A* and Mmr *(De Rossi et al 1999).*

1.5.3 Significance of MDR-TB

Isoniazid and Rifampicin, the two most potent antituberculous drugs, kill more than 99% of tubercle bacilli within 2 months of initiation of therapy *(Iseman and Madsen, 1989).* Isoniazid is critical early in therapy; its bactericidal activity rapidly reduces the sputum viable count because it is active mainly against the organisms growing aerobically in pulmonary cavities *(Drake 1999; Hobby 1952).*

Rifampicin is important in killing organisms that are metabolizing slowly, killing the persisters and sterilizing the patient's sputum *(Grumbach et al 1970).*

Along with these two drugs, Pyrazinamide, with a high sterilizing effect, appears to act on semi dormant bacilli not affected by any other antituberculous drugs. It is only active at low pH; making it ideally suitable for killing the organisms inside caseous necrotic foci explaining the finding that Pyrazinamide appears to have no benefit after the second month of therapy *(East African/British Medical Research Councils, 1981).*

Therefore, the emergence of strains resistant to these drugs causes major concern, as it leaves only drugs that are far less effective, have more toxic side effects, and result in higher death rates, especially among HIV-infected persons.

The **HIV epidemic** has completely destabilized the Tuberculosis control in high HIV prevalent regions. An estimated one third of the persons living with HIV infection are co infected with tuberculosis. HIV infection and MDR-TB was a perfect storm together and in 1993, WHO took an unprecedented step and declared Tuberculosis to be a global emergency.

Diabetic patients are more susceptible to have an aggressive course of tuberculosis. With regard

to the possible effects of DM on the outcome of TB, recent data are scarce. However studies show that they pose higher risk for spread of drug-resistant mycobacteria in the community. These issues require urgent attention.

Infections due to **Non tuberculous mycobacteria** are on the rise. They were not deemed significant pathogens until the mid 20th century with the emergence of pulmonary infections in patients with pre-existing lung diseases. Subsequently the AIDS epidemic has also brought forth drastic increase in NTM infections.

Human error is the principal factor associated with the emergence of drug resistant strains of *M.tuberculosis*. Prescription of inadequate chemotherapy, receiving improper treatment outside the National Programme from private qualified, or even unqualified practitioners, use of drugs of unproven bioavailability, patient's lack of knowledge of the treatment, difficulty experienced by poor patients due to lack of financial resources, shortages of drugs due to poor management and financial constraints in developing countries, poor case-management when the treatment is not directly observed are some of the reasons for the rise of multidrug resistance.

Today, with the greatly expanded efforts to strengthen tuberculosis prevention and control programmes worldwide, there is growing concern about the currently reported and potential future rates of drug-resistant tuberculosis. The resistant cases must be identified as swiftly as possible when they present at health care facilities so that they do not pose a threat to the community. To assess the extent of drug-resistant *M.tuberculosis* strains harbored among re-treatment pulmonary tuberculosis patients and analyse the factors that had contributed to it, the present study was undertaken at Tirunelveli Medical College.

Aim and Objectives

AIM AND OBJECTIVES

- To analyse the risk factors that had led to retreatment of the study group
- To study the profile of drug sensitivity pattern of CAT II patients and correlate it with the socio demographic status of these patients
- To assess the influence of risk factors like socio demographic characteristics, DM, HIV status, smoking and previous treatment as a marker for the development of Drug Resistant TB in the study group.

Review of literature

3. REVIEW OF LITERATURE

"I have no business to live this life if I cannot eradicate this horrible scourge from the mankind,"-Robert Koch, delivering a lecture at Berlin University on his discovery of tuberculosis bacilli, 1882.

It has been 125 years since Robert Koch first discovered the tuberculous bacilli and the world is still fighting hard to control the disease. The poor and developing countries are still in the grip of TB. Almost 40 years after introduction of combination chemotherapy for TB, and with the accumulated knowledge of the mechanisms leading to development of drug resistance, drug resistant TB, particularly MDR forms, remain a barrier to TB control.

3.1 The Global Burden of Tuberculosis

There were an estimated 9.2 million new cases of Tuberculosis in 2006 (139/ 1,00,000 population) including 4.1 million new smear-positive cases (44% of the total). India, China, Indonesia, South Africa and Nigeria rank first to fifth in terms of absolute numbers of cases. There was an estimated 4, 90,000 cases of multidrug-resistant Tuberculosis (MDR-TB) causing more than 1, 30,000 deaths. 1.7 million people died of Tuberculosis in 2006 including 2,31,000 people with HIV. This is equal to 4,500 deaths a day. By March 2008, XDR-TB cases had been confirmed in more than 45 countries and in all regions of the world. WHO estimates around 40,000 XDR-TB cases emerge every year *(WHO Tuberculosis fact sheet 2008).*

3.2 The Indian Scenario

In India, 1.8 million Tuberculosis cases occur annually, accounting for one-fifth of the world's new Tuberculosis cases and two-thirds of the cases in the South-East Asian region. This makes India the highest tuberculosis burden country in the world. It is estimated that two of every five Indians are infected with tuberculosis. Of them, at least 10% will develop Tuberculosis disease during their lifetime.

Around 0.8 million are sputum positive and one sputum positive patient can infect 10-15 persons in a year if left untreated. Tuberculosis is one of the leading causes of mortality in India, killing 2 persons every three minute, nearly 1,000 every day. The results of the Drug Resistant Surveys undertaken in Gujarat and Maharashtra (2005-2006) indicate prevalence of MDR-TB to be 3% amongst new cases and 12 - 18% in re-treatment cases. In India, XDR TB has been reported by isolated studies with non-representative and highly selected clinical samples. The magnitude of the problem remains to be determined due to the absence of laboratories capable of conducting quality assured second line Drug Sensitivity Test *(RNTCP Status Report 2008).*

3.3 The Global Prevalence of Drug Resistance The 2008 WHO/IUATLD Global Projects on Anti-Tuberculosis Resistance Surveillance report includes drug susceptibility test results from 91,577 patients from 93 settings in 81 countries and 2 Special Administrative Regions of China collected between 2002 and 2006. It is estimated that 489,139 cases emerged in 2006, and the global proportion of resistance among all cases is 4.8%. China, India and the Russian Federation are estimated to carry the highest number of MDR cases. China and India carry approximately 50% of the global burden and the Russian Federation a further 7%.

Countries of the former Soviet Union are facing a serious and widespread epidemic, that almost half of all TB cases are resistant to at least one drug and every fifth case of TB will have MDR-TB. MDR-TB cases in this region have more extensive resistance patterns including some of the highest proportions of XDR-TB.

China ranked second, but has the highest burden of cases in the world. It is estimated that over 1 in 10 cases of MDR TB that emerged in 2006 globally occurred in patients in China without a history of prior anti-TB treatment *(Anti-tuberculosis drug resistance in the world, WHO Fourth Global Report 2008).*

Compared with survey in 1997 at Poland, the 2000 survey showed no statistical difference in the rate of acquired resistance: 17.0% in 1997 and 16.6% in 2000. However, the MDR rate grew from

7.0% to 8.5% (22% increase) and 50% increase in patients excreting *M.tuberculosis* resistant to four drugs (1.4% in 1997 vs. 2.1% in 2000) *(Augustynowicz-Kopec et al 2000).*

From January 1991 to December 2000, a total of 291 HIV-negative patients treated at Muniz Hospital were affected by MDR-TB. Of these, 212 (72.9%) were acquired MDR-TB cases *(Domingo Palmero et al, 2003).*

Of the countries that reported data on drug resistance stratified by HIV status in the *WHO, Fourth Global Report 2008*, any resistance and MDR were significantly associated with HIV.

3.4 The Prevalence of Drug Resistance in India

Data from nine sites in India show that drug resistance among new cases is relatively low however, new data from Gujarat indicate that MDR is present in 17.2% among retreatment cases, which is higher than what was previously anticipated and it is estimated that 110,132 MDR-TB cases emerged in India in 2006, representing over 20% of the global burden *(Anti-tuberculosis drug resistance in the world, WHO Fourth Global Report 2008).*

A study conducted by the Institute of Thoracic Medicine, Chennai in four District Tuberculosis Centres of Tamil Nadu, showed that acquired resistance was
63 %, of which 23.5 % was resistance to a single drug and 39.5 % to more than one drug. MDR-TB was reported in 20.3 % *(Paramasivan CN, 1998).*

Acquired resistance to Isoniazid was 61.76%, to Streptomycin was 51.52%, to Rifampicin was 70.59% and to Ethambutol was 39.39%. Proportion of MDR- TB was 3.3% in new cases and 38.2% in old cases *(Mathur et al 2000).*

Of the patients with history of previous treatment, isolates from 50% were fully susceptible. Resistance to Isoniazid, alone or with other drugs was seen in 50% cases, Rifampicin resistance was observed in 25%, all of whom were also resistant to Isoniazid *(Paramasivan CN et al 2000).*

At Indore, Madhya Pradesh resistance for Isoniazid, Streptomycin and Pyrazinamide was found

to be high (54.2%, 41.5% and 50% respectively) followed by resistance to Rifampicin (25%) and Ethambutol (22%). Only 12% of the isolates were sensitive to all the anti-tuberculous drugs while resistance to two, three, and four or more drugs was in the range of 20-25%. MDR TB was 8.1% *(Hemvani et al 2001).*

In previously treated cases, resistance to any drug was observed in 81.2%, and any resistance to Isoniazid, Rifampicin and both in 81%, 69% and 69%, respectively in North Arcot (Tamilnadu). All previously treated patients were resistant to Isoniazid and Rifampicin (100%) in Raichur (Karnataka) *(Paramasivan CN et al 2002).*

Shah et al 2002 studied previously treated pulmonary Tuberculosis patients and reported that resistance to Isoniazid and Isoniazid plus Rifampicin was 12.86 and 15.77 % respectively.

Among the isolates from cases with previous history of treatment of varying duration, resistance to Rifampicin was 28.2% and to Isoniazid was 39.7%. 24.3% of these drug resistant isolates were multidrug resistant *(Malhotra et al 2002).*

In a study conducted by *Sophia Vijay et al 2002* in Bangalore, the multidrug resistance in previously treated cases was found to be 12.8 % and ranged from 8.4 to 17.2 %.

A retrospective study of drug resistance among treatment failure Tuberculosis cases observed a resistance of 42.5%. A high degree (14 %) of MDR-TB was observed. These patients claimed to have antituberculous therapy without improvement; however, 57.5% isolates were sensitive to all four first-line drugs that were tested *(Dam et al 2005).*

3.5 Potential Causes for Drug Resistance

Drug resistant tuberculosis is a man made problem. Various factors have been implicated in the causation of MDR-TB .They are

3.5.1. Genetic Factors

There is some evidence to postulate host genetic predisposition as the basis for the development of MDR-TB, though it has not been conclusive (*Weyer K Kleeberg 1992; Carpenter et al, 1983)***.** In a study from India *(Sharma et al 2003)*, patients with HLA-DRB1*13 and -DRB1*14 were found to have two-fold increased risk of developing MDR-TB. *Park et al 2002*, found that susceptibility to MDR-TB in Korean patients was strongly associated with HLA361 DRB1*08032-DQB1*0601 heliotypes.

3.5.2. Factors Related To Previous Anti Tuberculous Treatment

Incomplete and inadequate treatment:

The most powerful predictor of the presence of drug resistant tuberculosis is a history of prior treatment of Tuberculosis. Tuberculous patients in India get treated with DOTS regimen not only through RNTCP, but also receive treatment from private medical practitioners. Irregular, incomplete, inadequate treatment is the commonest means of acquiring drug resistant organisms *(Borgdorff et al 2002, Sharma & Mohan 2006, Vasanthakumari et al 1997).*

Mahmoudi and Iseman 1993, observed that errors in management decisions like addition of a single drug to a failing regimen, failure to identify preexisting drug resistance, initiation of an inadequate primary regimen, inappropriate Isoniazid preventive therapy and failure to identify and address noncompliance led to drug resistance.

A common error in prescription practice is the **"addition syndrome"**. If another drug is added to the existing regimen when the patient appears to deteriorate clinically and if resistance had developed to the drugs in use, adding another drug effectively amounts to monotherapy with the drug.

Prescription of antituberculous drugs by unqualified persons or alternative medicine practitioners in bizarre regimens for inadequate periods is an important problem in our country. Free availability of antituberculous drugs over the counter may contribute to this *(Sharma & Mohan 2004)*.

Inadequate treatment compliance:

Poor compliance with treatment is an important factor in the development of acquired drug resistance. In a study conducted in South India it was observed that only 43% of the patients receiving short-course treatment completed 80% or more of their treatment *(Datta et al 1993). Johnson et al 2003,* found a high incidence of drug resistance in previous treatment defaulters than in the new incident cases.

The studies on the association of demographic characteristics of patients to compliance of anti-tuberculosis therapy have given inconsistent results *(Sumartoyo et al 1993).*

An Indian study revealed that the socio-demographic factors like age, sex, education, occupation and socio-economic status were not associated significantly with adherence *(Pandit et al 2006).*

Ashry Gad et al 1997 in their study portrayed the same fact that the factors like age, sex, work and education had no association with adherence of treatment. However default was significantly more among male patients, alcoholics and smokers in several other Indian studies. (*Jaggarajamma et al 2007; Pauline Joseph et al 2006).* The studies by *Sophia Vijay et al 2003* and *Santha et al 2002* also suggest that males had twice the risk of treatment default than females. Better treatment compliance among women than men have been reported by *Ngamvithayapong-Yanai et al 1998* and *Balasubramanian et al 2004.*

Pronab Chatterjee et al 2003, observed that majority of patients on DOTS discontinued treatment because of toxicity of drugs or due to feeling better during treatment. Reasons for default from treatment like drug related problems, relief from symptoms, work related problems, treatment from other private or public health facility, domestic problems, stigma, too ill to attend, old age, other illnesses, migration, inconvenient DOT and dissatisfaction with treatment centre and DOT provider were observed in studies of *Sudha Ganapathy et al 1994*, *Jaggarajamma et al 2007* and *Sophia Vijay et al 2003*.

Various studies revealed that the compliance of DOTS was significantly high among those who have good knowledge about various aspects of disease *(Barnhoorn et al 1992; O'Boyle et al 2002; Thomas C, 2002).*The adequate knowledge about disease was found to be the protective factor from defaulting therapy in Ethiopia (*Tekle et al 2002)*. Increased probability of becoming infected with TB and developing active TB are associated with malnutrition, overcrowding, poor air circulation and poor sanitation- all factors associated with poverty *(Kamolratanakul et al 1999).* Studies from a number of developing countries reveal that the poor have much less access to DOTS programme than the non poor. A series of studies in India by *Pathania et al 1997* have strongly correlated income with TB. *Singh et al 2002, Balasubramanian et al 2000* and *Kemp et al 2001* observed that people living in poverty experience conditions that are more conducive to TB, have little access to health care, which delays diagnosis, and if they get treatment it is more likely to be inconsistent leading on to drug resistance. DOTS has the potential to reduce the economic and social burden of TB for patients and their households, however few studies have explicitly examined this question.

Bronchoalveolar macrophages among smokers contain high levels of iron, promoting the growth of *M.tuberculosis*. Iron loading causes reductions in TNF- α and nitric acid, which play a role in containing the intracellular growth of *M.tuberculosis (Boelaert et al 2003).* Studies by *Joanna d'Arc et al 2008, Thomas et al 2005* and *Kolappan et al 2002* support the observation that smokers are significantly more likely to relapse than nonsmokers. Similarly *Santha et al 2000,* observed an association between smoking and treatment failure and *Chandrasekaran et al 2005,* showed that smoking was a risk factor to default from treatment.

3.5.3 Lack of Laboratory Diagnostic Facilities

The importance of timely detection of drug resistance in the optimal management of patients with tuberculosis has not been fully recognized for many years. Good, reliable laboratory support is seldom available in developing countries. Unfortunately, these are the areas where MDR-TB is a major health hazard. When facilities for culture and sensitivity testing are not available, therapeutic decisions

are most often made by algorithms or inferences from previous treatment. Guidelines such as those published by the WHO are often resorted to choose the treatment regimen (*Crofton et al 1997).*

For patients categorized as treatment failure the WHO re-treatment regimen consists of three drugs (Isoniazid, Rifampicin, and Ethambutol) for a period of eight months, supplemented by Pyrazinamide during the first three months and Streptomycin during the first two months. If mycobacterial culture and *in vitro* sensitivity testing are not routinely performed, it is not possible to establish whether these patients are excreting multidrug resistant bacilli or not. If this WHO re-treatment regimen is administered to treatment failure patients who actually have MDR-TB, it is evident that during the last five months the patient will be receiving Isoniazid, Rifampicin and Ethambutol only and this would amount to **"monotherapy"** with Ethambutol. Thus, **"programmatic approach"** to the management of **"treatment failure"** patients may fail in some settings.

Coninx R et al 1999 assessed the programme of tuberculosis control using first line therapy and DOTS in a prison setting in Baku, Azerbaijan. Resistance to two or more drugs, a positive sputum result at the end of initial treatment, cavitary disease, and poor compliance were independently associated with treatment failure. The author concluded first-line therapy may not be sufficient in settings with a high degree of resistance to antibiotics.

Espinal et al 2000 made similar observations in another study with results of treatment with first line drugs for patients enrolled in the WHO and the IUATLD's global project on drug resistance surveillance. Patients with Tuberculosis in the Dominican Republic, Hong Kong Special Administrative Region (People's Republic of China), Italy, Ivanovoblast (Russian Federation), the Republic of Korea, and Peru were studied in this retrospective cohort study. The data suggested that standard short course chemotherapy, based on first line drugs, is an inadequate treatment for some patients with drug resistant Tuberculosis. Although the DOTS strategy is the basis of good Tuberculosis control, the strategy should be modified in some settings to identify drug resistant cases sooner, and to make use of second line drugs in appropriate treatment regimens.

3.5.4. Other Co-Morbid Conditions

While not the documented greatest risk for MDR-TB, co infection with HIV and Diabetes deserve special addressing.

HIV-TB: A Bidirectional Interaction:

HIV-infected persons are at markedly increased risk for progressive disease following primary tuberculous infection *(Liberato et al 2004).* HIV infection also increases the risk of subsequent episodes of Tuberculosis from exogenous reinfection *(Sonnenberg et al 2001, Small et al 1993).* The estimated annual risk of reactivation among those co-infected with HIV and TB is about 5 to 8 % with a cumulative lifetime risk of 30 % or more compared to HIV-negative adult patients *(Narain et al 1992).*

In early 1990s, several institutional outbreaks of MDR-TB among HIV-infected patients drew attention to the problem *(CDC Wkly Rep 1991; 1993; Fischl et al 1992).* MDR-TB has been shown to be almost twice as common in tuberculosis patients living with HIV compared to TB patients without HIV. However current evidence suggests that HIV infection *per se* does not appear to be a predisposing factor for the development of MDR-TB. The prevalent hypothesis is that HIV infection favours the transmission of multidrug resistant strains of *M.tuberculosis*.

Several factors such as

(*i*) Increased susceptibility to Tuberculosis

(*ii*) Increased opportunity to acquire Tuberculosis due to overcrowding, exposure to patients with MDR-TB due to increased hospital visits

(*iii*) Malabsorption of antituberculosis drugs resulting in suboptimal therapeutic blood levels in spite of strict adherence to treatment regimen

(iv) drug interactions between antiretrovirals and antimycobacterial agents, potentially increase the chances of MDR-TB in persons with HIV/AIDS *(Anti-tuberculosis drug resistance in the world, WHO Fourth Global Report 2008).*

Gordin et al 1996, New York, revealed that HIV infected TB patients were significantly more likely to develop resistance to at least one drug (37 versus 19%) and MDR (19 versus 6%) than those without HIV infection.

Kalpana et al 2004 observed that the level of MDR-TB in HIV positive was twice as high as in negative patients.

Among HIV-positive patients with a history of previous treatment, resistance was found to Isoniazid in 27% and to Rifampicin in 18.9% while MDR-TB was seen in 13.5% patients *(Swaminathan S et al 2005).*

Sarman Singh et al 2007 observed resistance to first-line drugs in 50% of the isolates of HIV-tuberculosis co-infection and 33.33% of the isolates were also resistant to second-line drugs.

Diabetes Mellitus and TB-Converging Epidemics:

Diabetes affects 230 million persons worldwide, and this number is anticipated to reach 366 million by 2030, at which time 80% of those affected will be living in low and middle income countries, where active tuberculosis is widespread *(Ruder et al 2007).* Eight of the 10 countries with the highest incidence of DM worldwide *(Wild et al 2004)* are also classified as high-burden countries for TB by the World Heath Organization *(WHO report 2007).* The consequences of these converging epidemics are likely to be substantial.

Diabetes mellitus predisposes to reactivation of tuberculosis infection. The relative risk of developing tuberculosis is up to five times higher in diabetics (*Kim et al 1995*). The reason for increased susceptibility of diabetics to TB may be due to

(i) Alveolar macrophages which are essential to eliminate mycobacterial infection are less activated in TB patients complicated with DM which may contribute to increased susceptibility *(Wang et al 1999).*

(ii) In a study of TB patients with DM a higher depression of cellular immunity was evidenced by fewer T lymphocytes and their decreased capacity for blast-cell transformation than those with TB

alone *(Karachunskii et al 1997).*

(iii) Interferon gamma production by CD4+ T-cell was reduced in patients with TB but those with poor diabetic control produced significantly less IFN-gamma than did patients with good diabetic control *(Tsukaguchi et al 2002).*

(iv) Changes in pulmonary vasculature and alveolar oxygen pressure may also be contributory *(Kameda et al 1986).*

Studies indicate that patients with TB who have DM present a higher bacillary load in sputum, delayed mycobacterial clearance and higher rates of multidrug resistant infection.This implies that patients with TB who have DM may be more seriously ill and may pose higher risk for spread of drug-resistant mycobacteria in the community.

3.6 Non Tuberculous Mycobacteria:

As routine drug sensitivity testing is not performed under the National control programmes, the non-responsive cases are usually labeled as MDR. Though development of drug resistance is a reason, the other equally important fact not given due importance is the occurrence of Non Tuberculous Mycobacteria which has intrinsic resistance for the standard anti-tuberculous drugs.

NTM, also known as atypical mycobacteria, are saprophytes naturally distributed in soil, water and dust. Their pathogenic potential has been recognized since the beginning of last century *(Duvall et al 1908).* These organisms have been reported to cause a variety of infections, more so in immuno compromised individuals. The incidence of tuberculosis has reduced in developed countries but infections due to NTM are on the rise *(Ferreira et al 2002)*, while in developing countries like India, tuberculosis is still a major health problem. Respiratory infections due to NTM are often associated with various conditions such as chronic obstructive pulmonary disease, cystic fibrosis of lung, bronchiectasis, emphysema of lung, previously treated pulmonary tuberculosis and lung cancer.

M.kansasi, M.scrofulaceum, M.fortuitum, M.avium complex, M.xenopi and *M.simiae have* been reported to cause pulmonary infections (*Katoch 2004). Jesudason et al 2005* reported that *M.chelonae*

and *M.fortuitum* accounted for 67% of NTM isolated from respiratory specimens. Since NTM are ubiquitous in nature and a possible laboratory contaminant, the isolation of these organisms from specimens should meet the criteria laid by the American Thoracic Society to confirm their etiological significance such as,

a) Repeated isolation of the same organism from a patient,
b) Associated positive clinical and radiological evidence and
c) Histopathological confirmation.

need to be considered while reporting NTM from clinical specimens. However, certain other parameters like

a) Collection of appropriate specimens directly from the lesion such as biopsies and BAL,
b) Isolation from sterile body fluids such as blood, CSF, pleural fluids,
c) Presence of any predisposing factors / underlying diseases and
d) The immune status of the patient

helps in assessing the etiopathogenesis of NTM when isolated *(Katoch 2004;Wallace et al 1990).*

Karak et al 1996 from Kolkata, have reported a NTM prevalence of 17.4% from sputum specimens from patients with fibrocavitary pulmonary diseases, this was comparatively higher than the reports of the other workers. *Chakrabarthi et al 1990* from Chandigarh documented NTM isolation rate of 7.4% from various clinical specimens and *M.fortuitum* was the commonest isolate. *Paramasivan et al 1985* from Chennai has reported 8.6% of NTM from sputum specimens of patients in BCG trial area and *M.avium / intracellulare* was the species most frequently isolated in their study. *Das et al 1982* reported isolation of 8.3% NTM from various clinical specimens from Delhi and Kasauli. These infections are under diagnosed in many laboratories due to lack of facilities and expertise. Regular documentation and reporting of these NTMs from clinical settings along with their sensitivity profiles is essential to be aware of the possible spectrum of diseases associated and preferred treatment options.

The rates of MDR-TB in previously treated cases vary from 6-60% in our country. This is a

matter of serious concern. Continuous monitoring of the trends of drug resistance is essential to assess the current interventions and their impact on the TB epidemic. Above all ensuring adherence to a full course of treatment is the key to cure TB patients and prevent the emergence of drug resistance.

Materials and Methods

4. MATERIALS AND METHODS

The present study was conducted at the Department of Thoracic Medicine, Tirunelveli Medical College, Tirunelveli for a period of one year from May 2007 to April 2008 to assess the drug susceptibility profile of Category II patients registered under RNTCP.

4.1 Study group

4.1.1 Inclusion criteria

The study population constituted

1. Smear positive patients, with history of previous anti tuberculous treatment for more than one month comprising cases of Failure, treatment after Default and Relapse started on the CAT-II regimen.
2. Patients who had completed or defaulted Category II treatment, found to be still sputum positive and referred to Tuberculosis Research Centre, Chennai for Drug Susceptibility Testing.
3. The above cases that had been previously treated under RNTCP were alone included.

4.2 Exclusion criteria

1. Extra pulmonary cases of Category II and
2. Cases who had been previously treated privately were not included for the study.

Socio demographic and clinical characteristics such as smear status, type of case, type of disease, category, treatment details such as drug regularity, number of doses taken by the patients and reasons for default were obtained from patient. Information on patient's literacy, occupation, and personal habits like smoking, other diseases like diabetes and HIV were also obtained.

4.3 Study Samples

Early morning sputum specimens were collected in a sterile container from the study group who were smear positive by Ziehl Neelsen method. Surface decontamination was done by immersing the specimen container in Lysol solution before transferring to the laboratory. All the laboratory works

were carried out as per standard laboratory procedures and Bio-safety norms in Class II Biosafety cabinet.

4.4 Acid-Fast Staining (Ziehl-Neelsen Method)

Mycobacteria retain the primary stain even after decolorizing with acid-alcohol, hence termed as 'acid fast'. A counterstain is employed to highlight the stained organisms for easier recognition. In Ziehl-Neelsen procedure, acid-fast organisms appear pink against a blue background.

4.4.1 Procedure

1. The slides were placed on a staining rack with the smeared side facing up.
2. Entire slide was flooded with strong carbol-fuchsin, which had been filtered before use.
3. Each slide was heated slowly until steaming, without allowing it to boil or dry. The steaming was maintained for five minutes by using intermittent heat.
4. Each slide was rinsed individually in a gentle stream of running water until all free stain was washed away.
5. The slides were then flooded with the decolorizing solution (20% sulphuric acid) for 2-3 minutes and rinsed thoroughly in a gentle stream of water to drain off excess stain.
6. The slides were then flooded with methylene blue counterstain for 30 seconds. Then the slides were rinsed thoroughly with water and smear was allowed to air dry.
7. The slides were examined under 100x objective lens, maintaining known positive slide as positive control and known negative slide as negative control.

4.4.2 Grading of AFB Smears by Z-N Microscopy in RNTCP

The smears were graded according to the RNTCP guidelines in Table - 4.1.

Table - 4.1 Grading of AFB Smears

No. of acid-fast bacilli	Fields	Report

(AFB)		
No AFB	In 100 immersion fields	**Negative**
1-9 AFB	In 100 immersion fields	**Record exact figure (1-9 AFB/ 100 fields)**
10 to 99 AFB	In 100 immersion fields	**1+**
1 to 10 AFB	Per field (examine 50 fields)	**2+**
More than 10 AFB	Per field (examine 20 fields)	**3+**

4.5 Culture of Sputum Specimens

The majority of clinical specimens submitted for tuberculosis culture laboratory are contaminated to varying degrees by more rapidly growing normal flora organisms. These would rapidly overgrow the entire surface of the medium and digest it before the tubercle bacilli start to grow. The specimens must, therefore, be subjected to a proper digestion and decontamination procedure that liquefies the organic debris and eliminates the unwanted normal flora.

4.5.1 Processing of Sputum Specimens by Sodium Hydroxide (Modified Petroff's Method)

1. To X ml of sputum, 2X ml of 4% Sodium Hydroxide (NaOH) was added and shaken with tightened cap and allowed to stand for 15 minutes at room temperature.
2. The specimen was centrifuged at 3000g for 15 minutes and the supernatant was poured off.
3. The sediment was resuspended in 20ml of sterile distilled water and centrifuged at 3000g for 15 minutes.
4. The supernatant was discarded and the sediment was inoculated in two slopes of Lowenstein Jensen medium in McCartney bottles.

4.5.2 Processing of Sputum Specimens Containing Cetyl Pyridinium Chloride (CPC) and Sodium Chloride (Nacl)

When the sputum specimens could not be cultured on the same day, they were processed within a week by CPC method.

1. To the specimen with equal amount of CPC/NaCl, 15-20ml of sterile distilled water was added to reduce the viscosity.
2. Cap was tightened to the container and mixed well by inversion and was centrifuged at 3000g for 15 minutes. The supernatant was discarded.
3. The sediment was resuspended in 20ml of sterile distilled water and was centrifuged at 3000g for 15 minutes.
4. The supernatant was decanted and the deposit was inoculated onto two LJ slopes.

4.6 Culture Examination

Tubercle bacilli do not grow in primary culture in less than one week and usually require two to four weeks to give visible growth from sputum specimens. Typical colonies of *M. tuberculosis* are rough, crumbly, waxy, non-pigmented (buff colored) and slow-growers having the appearance of breadcrumbs or cauliflower.

4.6.1 Examination Schedule

All cultures were examined within 48-72 hours after inoculation to detect gross contaminants. Thereafter cultures are examined weekly, up to 8 weeks on a specified day of the week. During examination, slopes in which the surface has been completely contaminated or where the medium has been liquefied or discoloured were discarded.

4.6.2 Reading of Cultures, Recording and Reporting of Results

All the slopes were held at 45° under tungsten filament lamp to observe the colony morphology clearly.

- All slopes that have completed 8 weeks of incubation and showed no growth were reported as 'negative'.
- All positives, Non Tuberculosis Mycobacteria or contaminated cultures were taken for further examination.
- Final results recorded: Negative, Contaminated and if positive the degree of positivity.

4.6.3 Reporting Of Positive, Negative and Contamination Results

The culture results were reported as per Table - 4.2 and 4.3.

- If the degree of positivity was different on the two slopes, the highest degree was recorded.
- If neither of two slopes showed 20 colonies individually but the total on both slope was more than 20 colonies it was 1+.
- If sum of colony was less than 20 on both slopes the actual number of colonies, was recorded.
- If one slope was positive and the other was negative the culture was reported positive with degree of positivity recorded and the slope showing no growth was re-incubated.
- If one slope was contaminated final result depended on the final examination of the remaining slope.
- If one slope showed the presence of NTM and the other slope was negative the final result was Negative / NTM.
- If both the slopes produced NTM at different weeks, the first NTM result in the final result column with the degree of growth was taken.
- If both the slopes showed NTM at the same week it was proceeded as for M. tuberculosis (grading).

4.7 Biochemical methods for identification of mycobacteria

Identification of Mycobacteria species requires a battery of biochemical tests. The following tests, when used along with the morphological characteristics, will enable a precise identification of

more than 95% of the *M.tuberculosis* strains.

1. Susceptibility to p-nitrobenzoic acid (PNB)
2. Niacin production test

Table - 4.2 Reporting of Cultures

READING	REPORT
No growth	Negative
1-19 colonies	Positive (number of colonies)
20-100 colonies	Positive (1+)
>100 discrete colonies	Positive (2+)
Confluent growth	Positive (3+)
Contaminated	Contaminated

Table - 4.3 Reporting of Positive Culture Results

SLOPE 1	SLOPE 2	
3+	2+/ 1+ / No growth /NTM	3+
2+	1+ / No growth / NTM	2+
1+	<20 Colonies / No growth / NTM	1+
< 20	<20	1+ (if sum of both slope is ≥20)
< 20	< 20	Actual number (if sum of both slope is < 20)
<20	*	Actual colony count
<20 NTM		Actual colony count
<20 No growth		Actual colony count
*	*	Contamination
*	No growth	Negative

*	NTM	NTM
No growth	No growth	Negative
No growth	NTM	Neg/ NTM
NTM	NTM	NTM (colonies or 1+ or 2+ or 3+)

* Contamination

4.7.1 Susceptibility to p-nitrobenzoic acid (PNB)

The species *of M.tuberculosis* complex are susceptible to PNB (500µg /ml), whereas the NTM are resistant to PNB.

1. The neat bacterial suspension were inoculated onto two slopes of LJ medium without drugs and one slope of LJ medium containing p-nitrobenzioc acid (PNB) at a concentration of 500mg/litre and incubated at 37°C.
2. Results read after 28 days.

M.tuberculosis does not grow on PNB medium. All other mycobacteria are resistant.

4.7.2 Niacin Production Test

Niacin plays a vital role in oxidation reduction reactions that occur during metabolic synthesis in all Mycobacteria. It functions as a precursor in the biosynthesis of co-enzymes. Although all mycobacteria produce Nicotinic acid, comparative studies have shown that, *M.tuberculosis* accumulates the largest amount and detection of this accumulated Niacin is useful for the definitive diagnosis of this species.

Reagents:

- O-toluidine 1.5%.

- Cyanogen bromide solution, approx. 10%.

Procedure:

1. All the LJ slopes were checked for water of condensation in culture tube, when needed 1 ml of sterile distilled water was added.
2. The culture bottles were then autoclaved for15 minutes at 121° C.
3. The bottles were placed in upright position for 20 - 30 minutes to allow the fluid to drain to the bottom.
4. 0.25 ml of autoclaved culture extract was pipetted out into a clean screw capped tubes.
5. Sequentially 0.25ml of O-toluidine and 0.25ml of cyanogen bromide was added and mixed well.
6. The tubes were closed and solution was observed for the formation of pink color (positive) within five minutes.
7. H37RV as positive control and MAC as negative control were also maintained.

4.8 Drug Susceptibility Testing

The drug sensitivity testing for the positive cultures were carried out at Tuberculosis Research Centre (ICMR), Chetput, Chennai.

The inoculum was prepared by using a representative sweep of the entire surface of the growth on the slope. The absolute concentration method uses a standardized inoculum grown on drug-free media and media containing graded concentrations of the drugs to be tested. Resistance is expressed in terms of the lowest concentration of the drug that inhibits growth; i.e., minimal inhibitory concentration (MIC).

4.8.1 Procedure:

Sterile distilled water 0.2ml was added to sterile bijou bottle with 10-12 glass beads (3mm diameter). Using a 3 mm internal diameter 24 SWG wire loop, two thirds of a loopful of representative

sample of the bacterial mass that approximately equaled to 4 mg moist weight was taken and delivered into the bijou bottle. Bottle was vortexed for 30 to 60 seconds at a speed which will just lift the beads from the bottle and produce uniform suspension. 0.8ml sterile distilled water was added and the bottle was shaken by hand and the suspension was left inside the cabinet for 15-20 minutes, for the coarser particles settle down. Using a 3mm external diameter 27 SWG nichrome wire loop one loopful of this suspension was inoculated in two drug free slope (control) and on drug containing slope of each concentration of the drug(s) and also one slope containing p-nitro-benzoic acid (500µg/ml) for each strain tested. The various drug concentrations used for absolute concentration method of DST are listed in Table - 4.4.

4.8.2 Incubation and reading of the tests:

The inoculated slopes were incubated at 37 ° C and examined for growth after 28 days of incubation.

- The lowest concentration of the drug inhibiting growth was recorded (MIC). The MIC for the drugs is given in Table - 4.5.
- In this method 'growth' is defined by the presence of 20 colonies or more. The test strain was considered to be resistant to the particular drug if the culture was positive above the MIC of each drug. The results were recorded as in Table - 4.6.

All the above procedures were done as per the following guidelines.

1. RNTCP Training Module for Medical Practitioners, Central TB Division Directorate General of Health Services, Ministry of Health and Family Welfare, Nirman Bhawan, New Delhi, 2006.
2. Canetti, G, Fox W, Khomenko A, Mahler, H.T., Menon, N.K., Mitchison.D.A Advances in techniques of testing mycobacterial drug sensitivity and the use of sensitivity tests in tuberculosis control programmes. Bull WHO, 1969; 41, 21-43
3. National Committee for Clinical Laboratory Standards. Susceptibility testing of Mycobacteria, Nocardiae and other aerobic Actinomycetes. Approved Standard. Wayne, PA: NCCLS; 2003.

Table - 4.4 Drug Concentrations (µg/ml)

S.NO.	DRUG	H37RV	TEST STRAIN
1.	STREPTOMYCIN	2,4,8,16,32,64	8,16,32,64
2.	ISONIAZID	0.025,0.05,0.1,0.2,1, 5	0.2,1,5
3.	RIFAMPICIN	4,8,16,32,64,128	32,64,128
4.	ETHAMBUTOL	0.5,1,2,4,8	2,4,8
5.	KANAMYCIN	2,4,8,16,32,64	8,16,32,64
6.	ETHIONAMIDE	20,28.5,40,57,80,114	20,28.5,40,57,80,114
7.	OFLOXACIN	0.5,1,2,4,8	2,4,8

Table - 4.5 Minimum Inhibitory Concentration

SL.NO	DRUG	MIC(conc. of drug)
1.	Streptomycin	8
2.	Isoniazid	5
3.	Rifampicin	128
4.	Ethambutol	8
5.	Kanamycin	64
6.	Ethionamide	114
7.	Ofloxacin	8

Table - 4.6 Grading of Culture slopes

GRADING	CULTURE GROWTH
3+	Confluent growth
2+	Innumerable colonies (>100 colonies)
1+	20-100 colonies
1-19	Actual number of colonies

Results

5. RESULTS

5.1 The Study Group

A total of 108 Category II tuberculosis cases were included for this study. These included patients residing in Tirunelveli district who were registered as Category II (Default, Failure & Relapse) under RNTCP at the Department of Thoracic Medicine, Tirunelveli Medical College Hospital, Tirunelveli and patients who had failed Category II and referred to Tuberculosis Research Centre, Chennai from Tirunelveli for further management. The period of study was one year from May 2007 to April 2008.

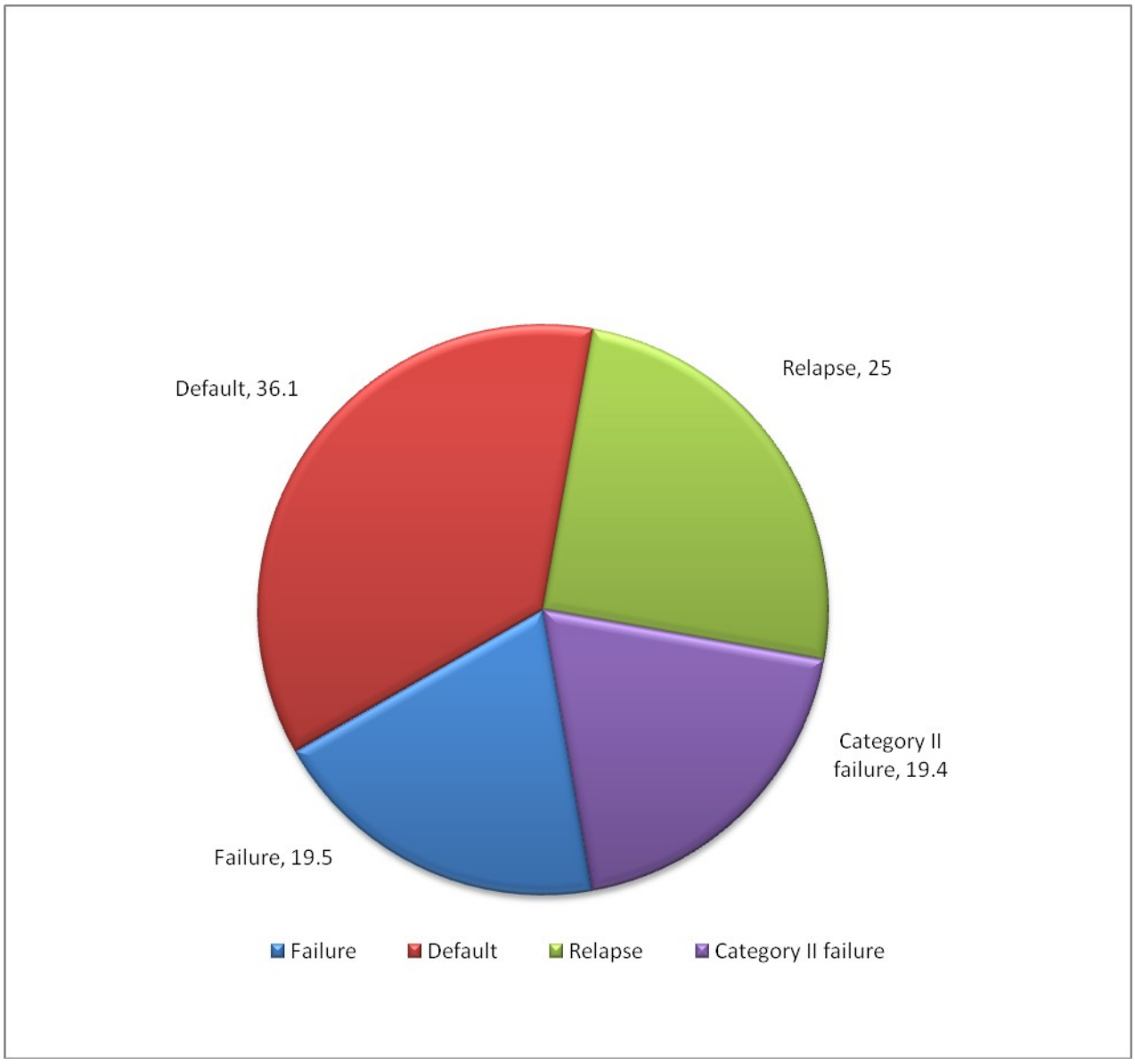
Table – 01 Types of patients selected in the study group.

Sl.No.	CASES	NO. OF PATIENTS	PERCENTAGE
1	FAILURE	**21**	**19.5**
	CATEGORY I	19	17.4
	CATEGORY III	2	1.9
2	DEFAULT	**39**	**36.1**
3	RELAPSE	**27**	**25.0**
4	CATEGORY II FAILURES	**21**	**19.4**
	TOTAL	**108**	**100%**

5.2 Statistical Analysis

The collected data were edited for completeness, consistency and accuracy. They were analysed by the parameters like mean, median and percentages. The differences of above parameters were tested by the parametric tests like 'Z' and't' and non-parametric test like χ^2 test, which was applicable wherever. The statistical package used for analysis and interpretation is SPSS (version-13) with the level of significance P=0.05.

Fig. 01. Types of Patients Selected in the Study Group (in Percentage).



5.3 Analysis of risk factors for retreatment

5.3.1 Age and Sex

The selected 108 study subjects were analysed based on age and sex. The results of the analysis are tabulated in Table - 02 and Fig. 02.

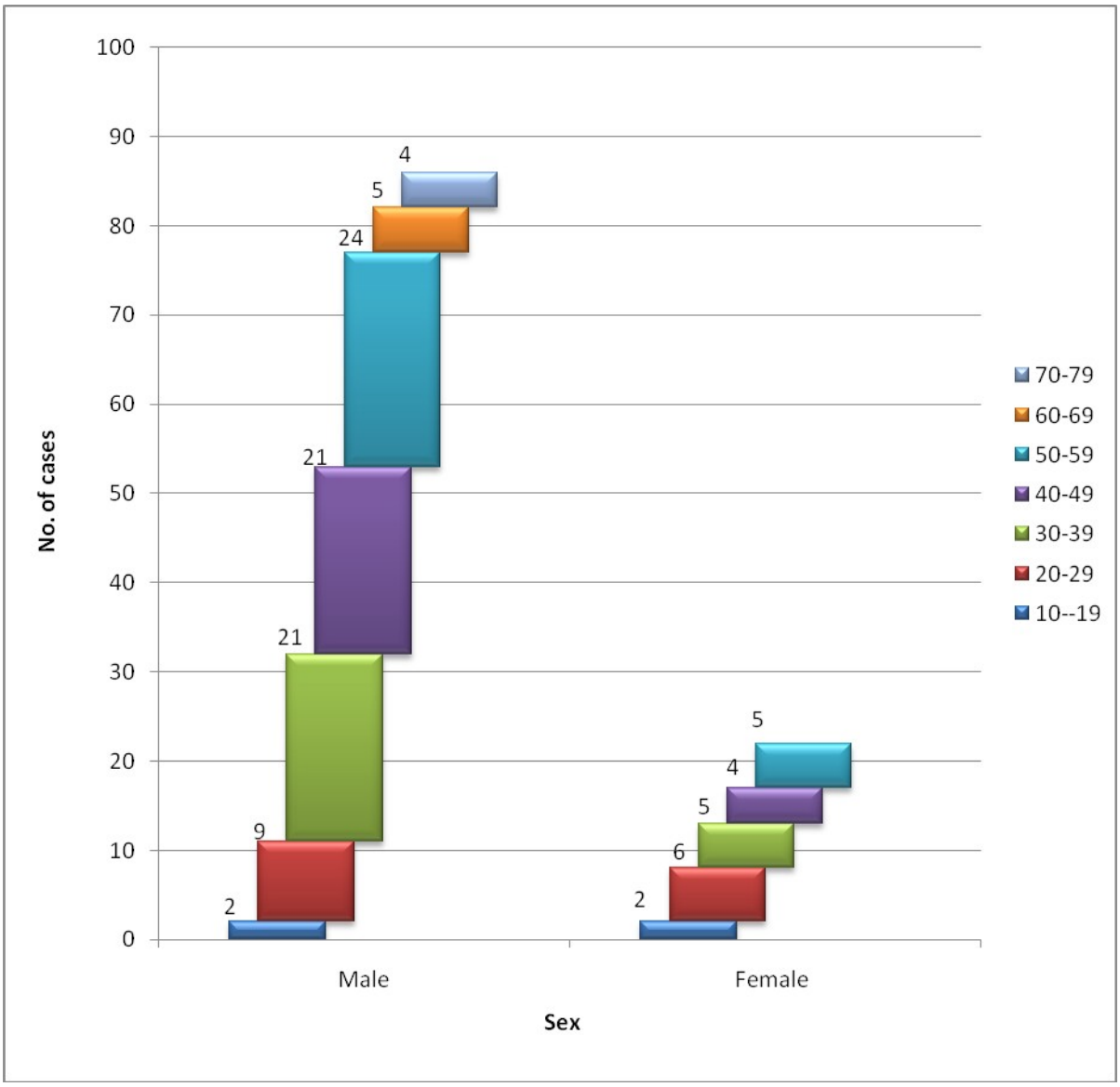
Table - 02. Age and sex wise distribution of the study subjects.

Sl. No	**Age group**	**Male**	**Female**	**Total**
1	10-19	2	2	4
2	20-29	9	6	15
3	30-39	21	5	26
4	40-49	21	4	25
5	50-59	24	5	29
6	60-69	5	0	5
7	70-79	4	0	4
Total		86	22	108
Mean		44.4	37.1	42.9
S.D		13.3	12.3	13.1
't'		2.44		
Significance		**P<0.05**		

The analysis shows that the mean age of female is 37.1±12.3 and of male is 44.4 ±13.3.

The numbers of male patients in each age group was proportionately higher than that of female patients except in the age group of 10-19 and 60-79. It was highest in the age group 30-59 years.

Fig. 02. Age and Sex wise distribution of the study subjects.



The sex wise distribution of cases under study is tabulated in Table - 03 and Fig. 03.

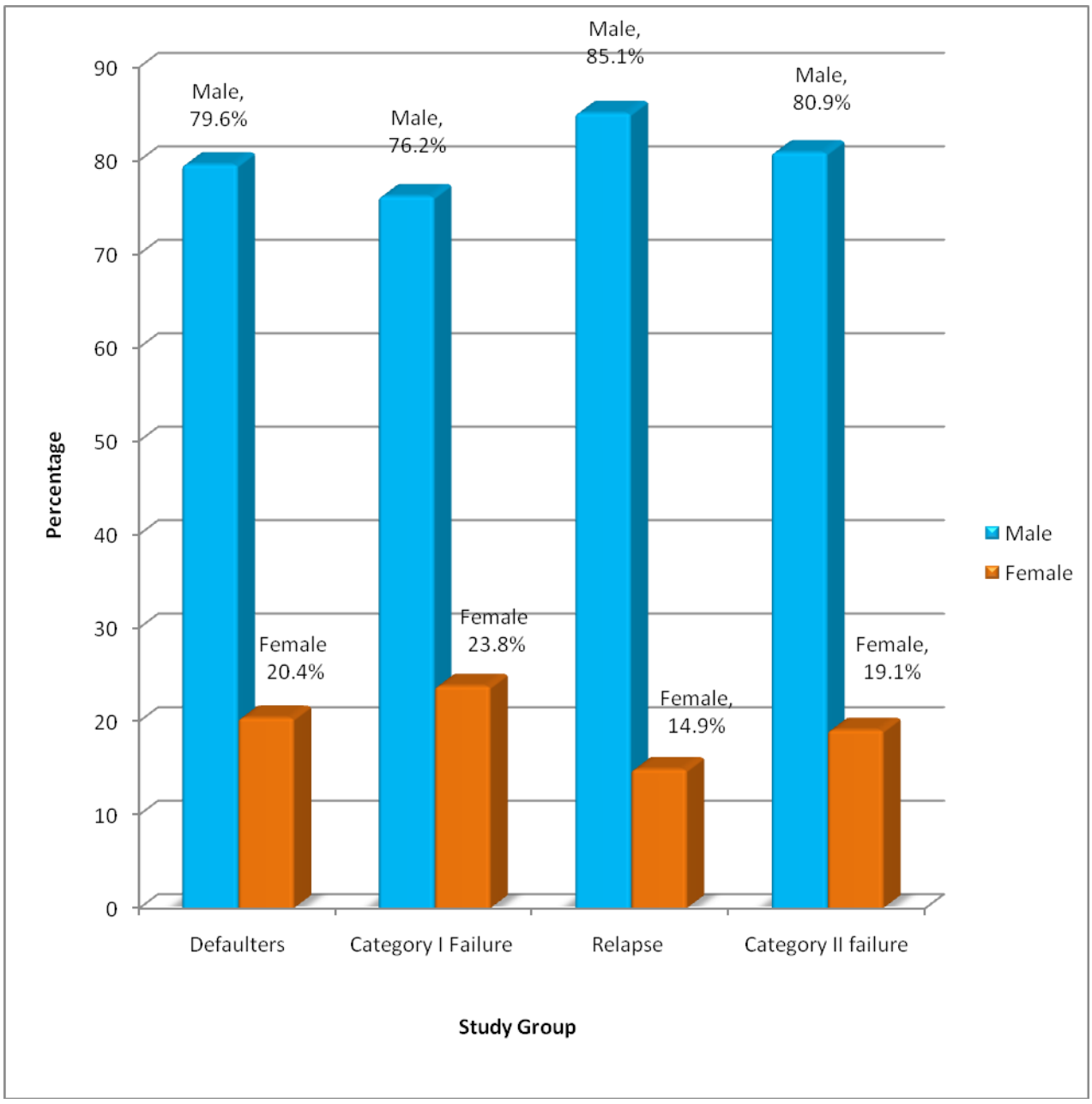
Table – 03 Sex wise distribution of cases under study.

Sl. No	**Study group**	**Male**		**Female**		**Total**		**Significance**
		No.	%	No.	%	No.	%	
1	Defaulters	30	34.9	9	40.9	39	36.2	$p > 0.05$
2	Category I Failure	16	18.6	5	22.7	21	19.4	$p > 0.05$
3	Relapse	23	26.7	4	18.2	27	25	$p > 0.05$
4	Category II Failure	17	19.8	4	18.2	21	19.4	$p > 0.05$
	Total	86	100	22	100	108	100	

Among the 108 cases, 86 were male (79.6%) and 22 were female (20.4 %). Of the 39 defaulter cases included under the study 30 were male (76.9%) and 9 were female (23.1%). Among the 21 Cat I failure cases 16 were male (76.2%) and 5 were female (23.8%). Of the total 27 relapse cases, 23 were male (85.1%) and 4 were female (14.9%). Among the 21 Category II failures who were referred to Chennai, 17 were male (80.9%) and 4 were female (19.1%).

However in comparison to the total male and female cases included under the study, there was no significance observed in relation to sex and each of the four study groups.

Fig. 03. Sex wise distribution of cases under study.


Male, 79.6%
Female 20.4%
Male, 76.2%
Female 23.8%
Male, 85.1%
Female, 14.9%
Male, 80.9%
Female, 19.1%
90
80
70
60
50
40
30
20
10
0
Percentage
Defaulters
Category I Failure
Relapse
Category II failure
Study Group
Male
Female

5.3.2 Residence

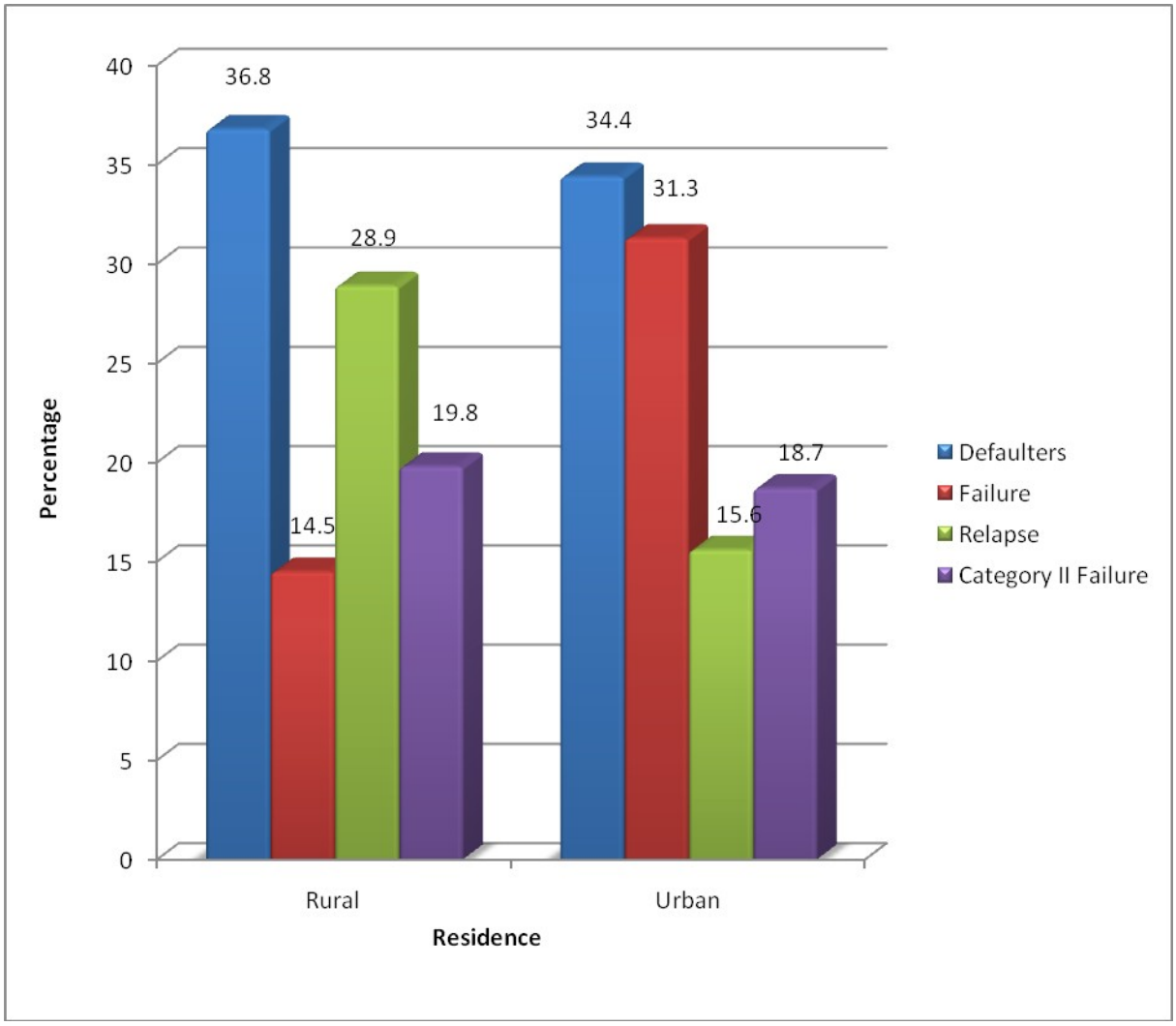
Patients who resided within the Tirunelveli Corporation limits were taken as urban population and the others as rural population. (Table - 04, Fig. 04)

Table - 04. Residence wise distribution of the study group.

Sl. No	Study group	Rural		Urban		Total		Significance
		No.	%	No.	%	No.	%	
1	**Defaulters**	28	36.8	11	34.4	39	36.2	$p > 0.05$
2	**Failure**	11	14.5	10	31.3	21	19.4	$p > 0.05$
3	**Relapse**	22	28.9	5	15.6	27	25	$p > 0.05$
4	**Cat II failure**	15	19.8	6	18.7	21	19.4	$p > 0.05$
	Total	76	100	32	100	108	100	

The defaulters of rural and urban residents are 36.8% and 34.4% respectively. The failure subjects of category I in rural and urban area are 14.5% and 31.3% respectively. The relapse cases of rural and urban area are 28.9% and 15.6% respectively. The Category II failure cases of rural and urban area are 19.8% and 18.7% respectively. The percentages are not statistically significant from the above results

Fig. 04. Residence wise distribution of the study group.



5.3.3 Income

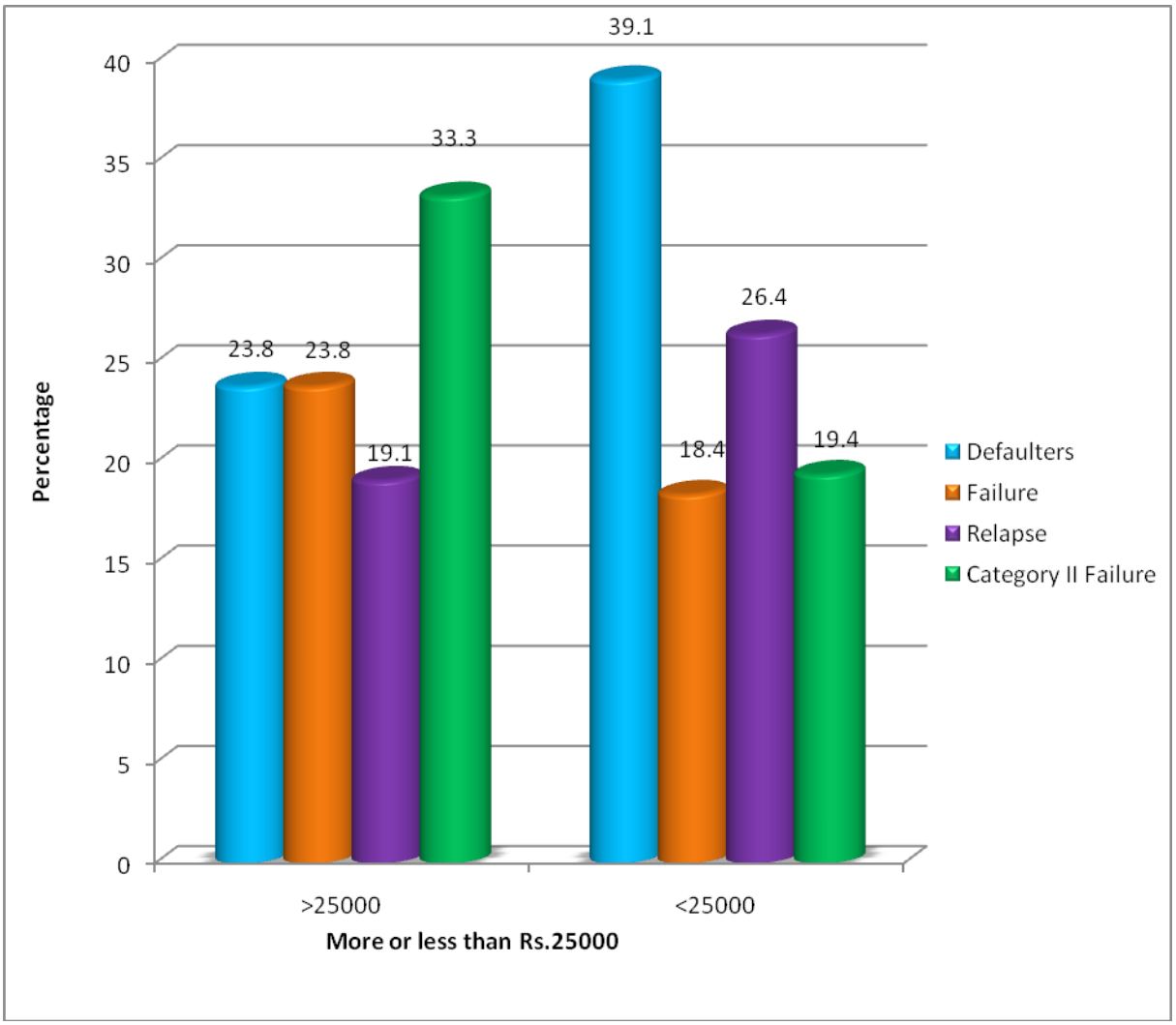
The average yearly income as stated by the patients was analysed (Table - 05, Fig. 05).

Table - 05. Income wise distribution of the study group.

Sl. No	Study group	More than 25000 as yearly ncome.		Less than 25000 as yearly income		Total		Significance
		No.	%	No.	%	No.	%	
1	**Defaulters**	5	23.8	34	39.1	39	36.2	p > 0.05
2	**Failure**	5	23.8	16	18.4	21	19.4	p > 0.05
3	**Relapse**	4	19.1	23	26.4	27	25.0	p > 0.05
4	**Cat II failures**	7	33.3	14	16.1	21	19.4	p > 0.05
	Total	21	100	87	100	108	100	

Only 21 cases had stated that their yearly income was above Rs. 25,000 (19.4%). The remaining 80.6 % of cases earned less than Rs. 25,000 only and were considered as living below the poverty line for analysis. Though a significant percentage of cases in each of the study group lived below the poverty line, an association was not observed.

Fig. 05. Distribution of study subjects and their income.



5.3.4 Education

Persons were considered as

Uneducated - Illiterate (unable to write their name)

Literate (able to read and write, but having concluded less than the high school level)

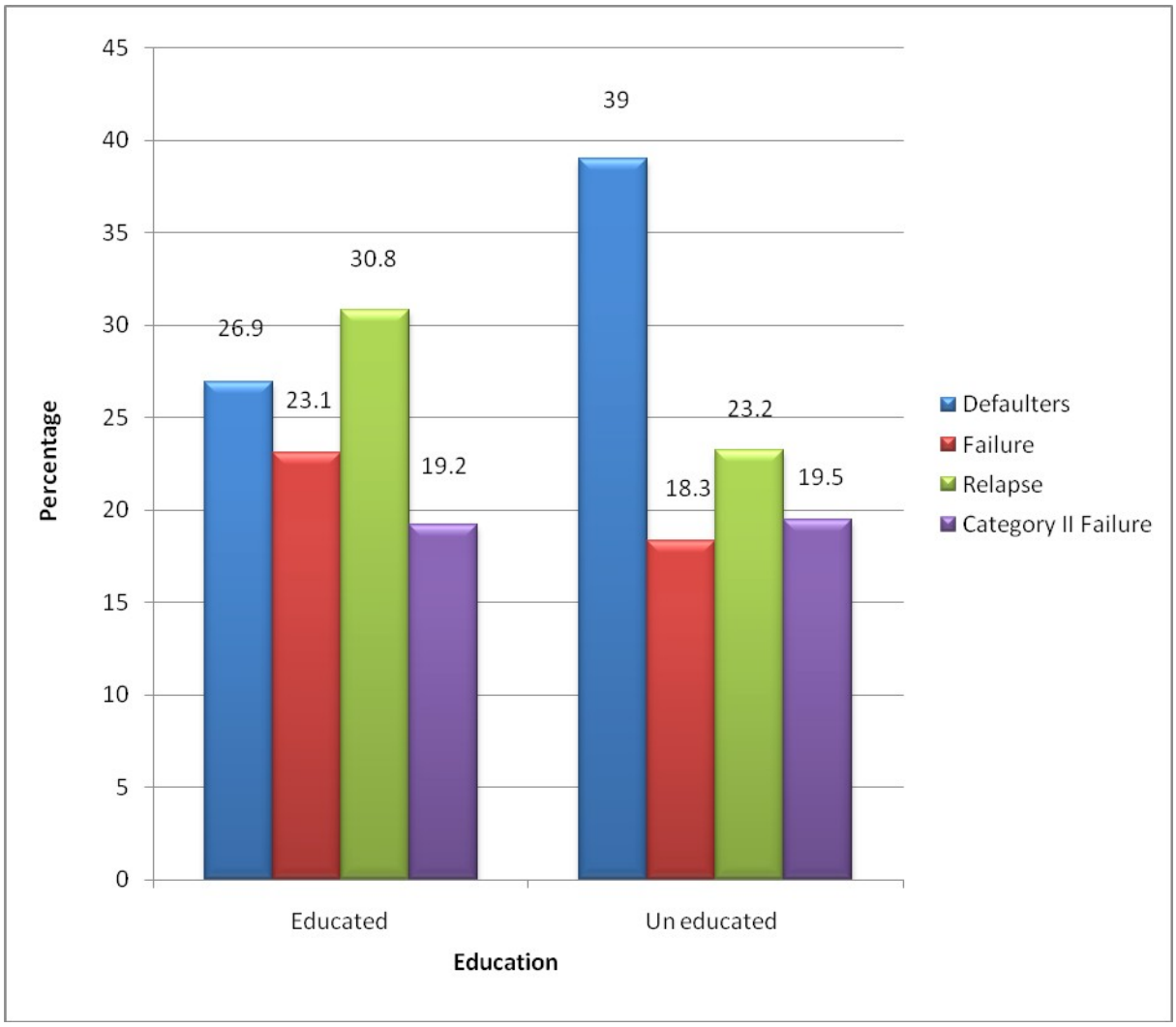
Educated - Above the high school level.

Their relation to the study group was analysed (Table - 06, Fig. 06). Among the 108 cases, 26 cases were considered educated as they had a minimum high school level of education (24%). The remaining 82 cases were uneducated (76%). There was no significant difference among the educated and uneducated in each of the study group.

Table - 06. Education wise distribution of the study group.

Sl. No	Study group	Educated		Un-educated		Total		Significance
		No.	%	No.	%	No.	%	
1	**Defaulters**	7	26.9	32	39.0	39	36.2	$p > 0.05$
2	**Failure**	6	23.1	15	18.3	21	19.4	$p > 0.05$
3	**Relapse**	8	30.8	19	23.2	27	25.0	$p > 0.05$
4	**Cat II failures**	5	19.2	16	19.5	21	19.4	$p > 0.05$
	Total	26	100	82	100	108	100	

Fig. 06. Distribution of study subjects and their Educational Status.


45
40
35
30
25
20
15
10
5
0
Percentage
26.9
23.1
30.8
19.2
39
18.3
23.2
19.5
Educated
Un educated
Education
Defaulters
Failure
Relapse
Category II Failure

5.3.5 Smoking Habit

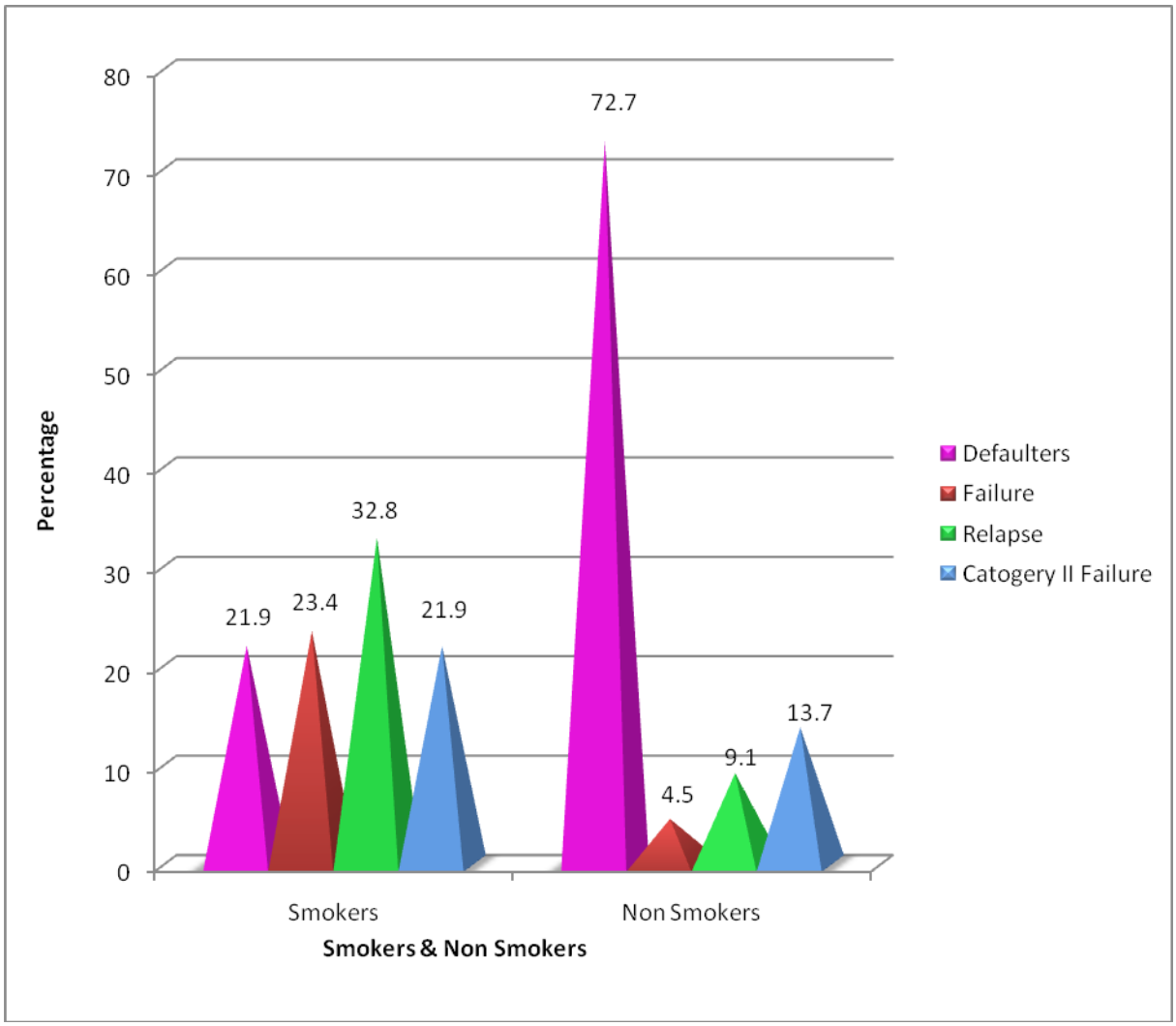
Patients who gave a history of habitual smoking during the previous treatment but had stopped at present and who were currently smoking were considered smokers for the purpose of the analysis (Table - 07, Fig. 07). In this study only male subjects had the habit of smoking, so the analysis is ear marked to male subjects. Out of the 86 male cases, 64 were smokers (74.4%) and 22 were non smokers (25.6%).

Smoking habits among Defaulters, Failure cases, Relapse cases and Cat II failures were analysed. It was found that smoking was a significant risk factor among Relapse cases, Category I and Category II failures.

Table – 07. Smoking habit as a risk factor among the study group.

Sl. No	**Study group**	**Smokers**		**Non smokers**		**Total**		**Significance**
		No.	%	No.	%	No.	%	
1	**Defaulters**	14	21.9	16	72.7	30	34.9	p <0.05
2	**Failure**	15	23.4	1	4.5	16	18.6	**p<0.01**
3	**Relapse**	21	32.8	2	9.1	23	26.7	**p<0.01**
4	**Cat II failures**	14	21.9	3	13.7	17	19.8	**p < 0.05**
	Total	64	100	22	100	86	100	

Fig. 07. Smoking habit as a risk factor among the study group.



5.3.6 Diabetes

History of Diabetes mellitus in the study group was analysed (Table - 08, Fig. 08).

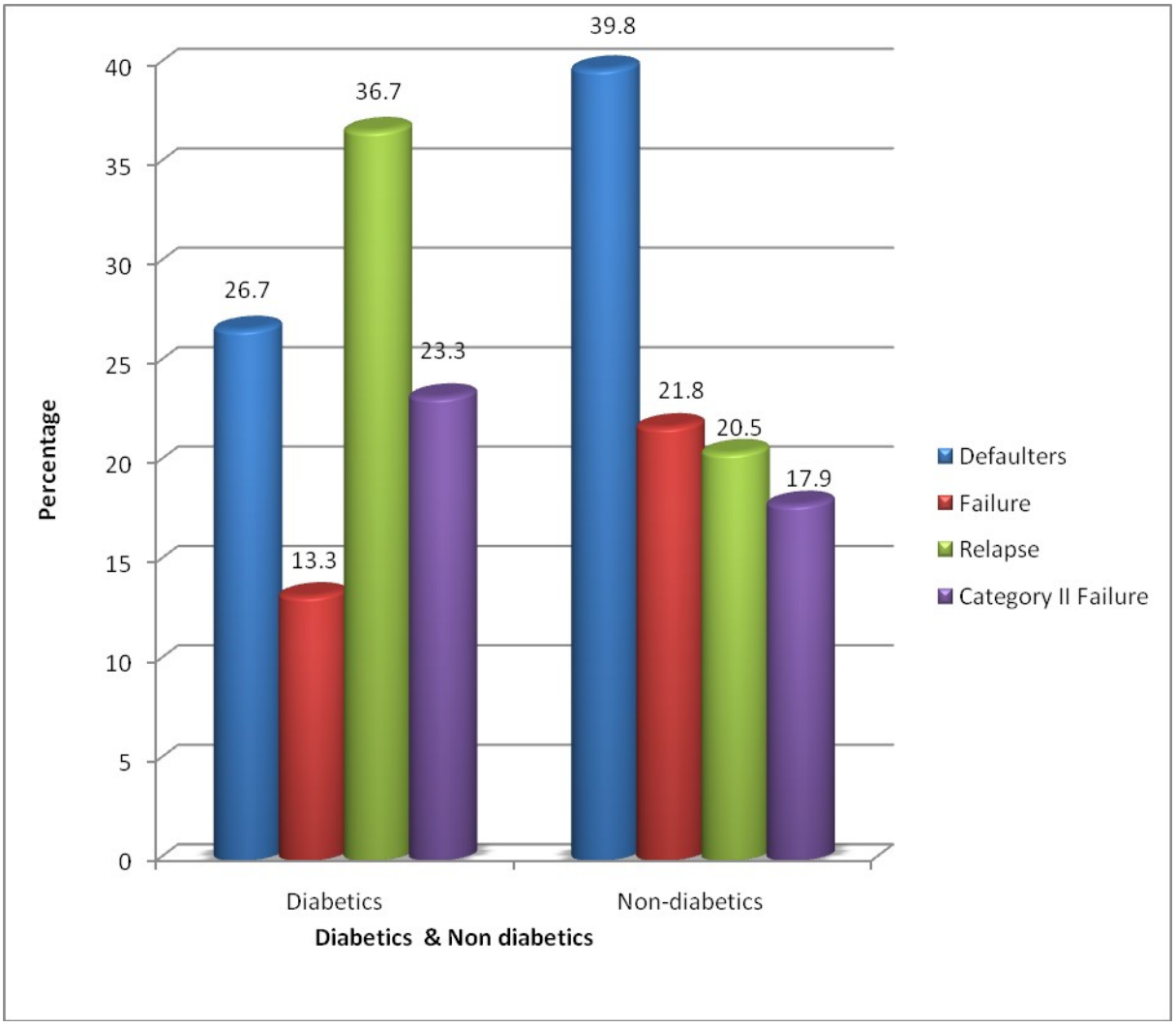
Table – 08. Diabetes as a risk factor among the study group.

Sl. No	Study group	Diabetics		Non diabetics		Total		Significance
		No.	%	No.	%	No.	%	
1	**Defaulters**	8	26.7	31	39.8	39	36.2	p >0.05
2	**Failure**	4	13.3	17	21.8	21	19.4	p>0.05
3	**Relapse**	11	36.7	16	20.5	27	25.0	p>0.05
4	**Cat II failures**	7	23.3	14	17.9	21	19.4	p > 0.05
	Total	30	100	78	100	108	100	

Of the 108 patients, 30 patients were on treatment for diabetes. 9 patients were newly detected as diabetics based on the fasting blood sugar level of more than 140 mg/dl and postprandial level more than 180 mg/dl. Those who were on prior oral hypoglycemic drugs had moderate glycemic control with average serum glucose levels between 200 mg/dl and 300 mg/dl. The duration of diabetes, at the time tuberculosis was diagnosed varied from 1 year to long-standing disease of 10 years (average 6.4 years).

In the study group 30 cases (27.7%) were diabetic. There were more number of non-diabetics in the entire study group except among Category II failures, where 14 cases (66.6%) were found to be diabetic. There was no significance observed in relation to diabetic status and each of the four study groups.

Fig. 08. Diabetes as a risk factor among the study group.



5.3.7 Regularity of Treatment

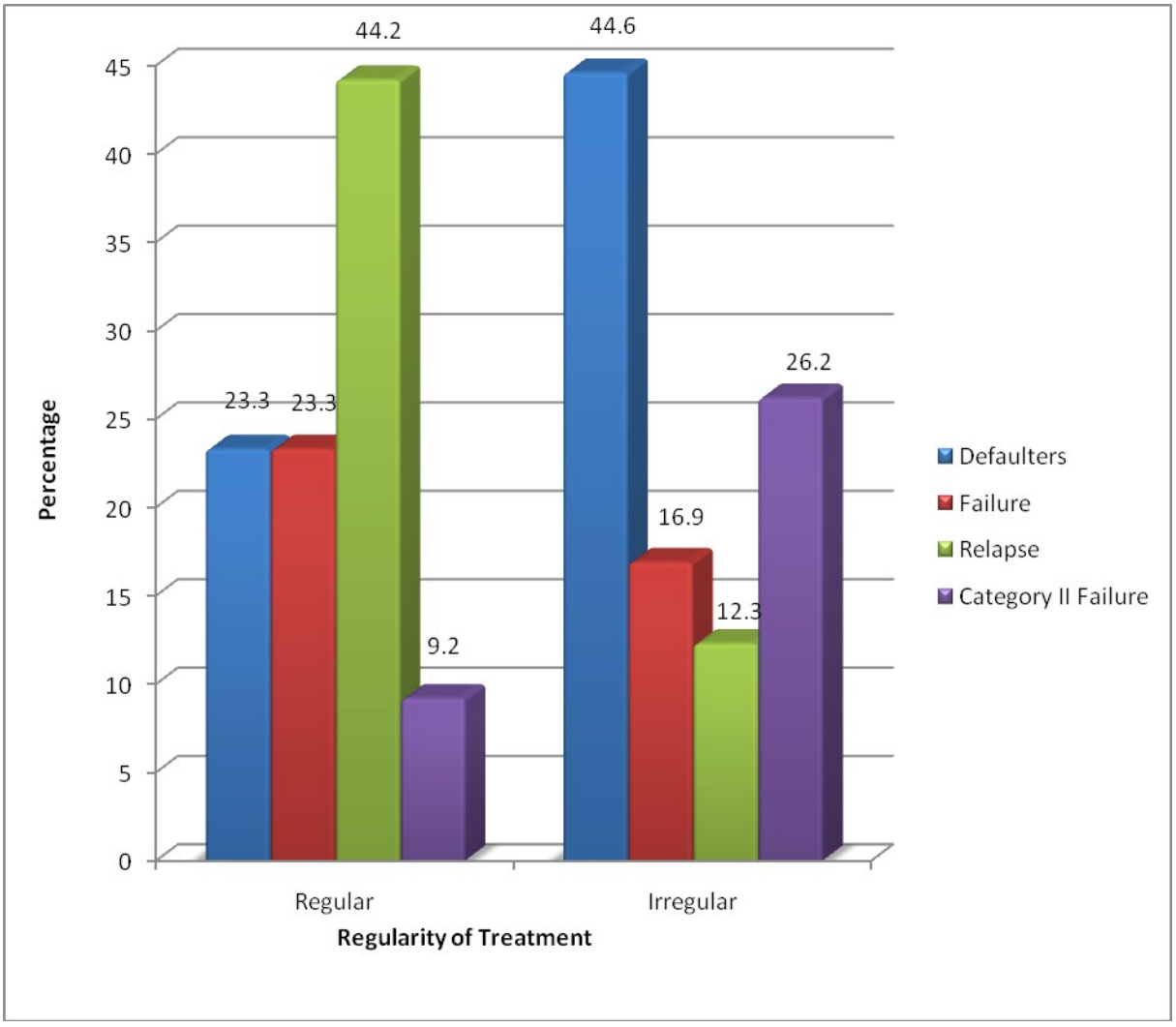
Treatment was considered as being on a regular basis if the medication was used as prescribed. Treatment was considered irregular if there was default in the use of medication for five or more consecutive doses provided it did not reach 30 days a month. The regularity of treatment was assessed and analysed for the study subjects (Table - 09, Fig. 09).

Table – 09. Regularity of treatment among the study group.

Sl. No.	Study group	Regular		Irregular		Total		Significance
		No.	%	No.	%	No.	%	
1.	**Defaulters**	10	23.3	29	44.6	39	36.2	**P < 0.01**
2.	**Failure**	10	23.3	11	16.9	21	19.4	P > 0.05
3.	**Relapse**	19	44.2	08	12.3	27	25.0	P >0.05
4.	**Category II failure**	4	9.2	17	26.2	21	19.4	**P < 0.05**
	Total	43	100	65	100	108	100	

Only 40% of the total cases had taken a regular treatment previously and the remaining 60% admitted that they had not strictly adhered to the treatment schedule. The percentages of patients on irregular treatment are statistically significant among defaulters and Category II failure cases.

Fig. 09. Regularity of treatment among the study group.



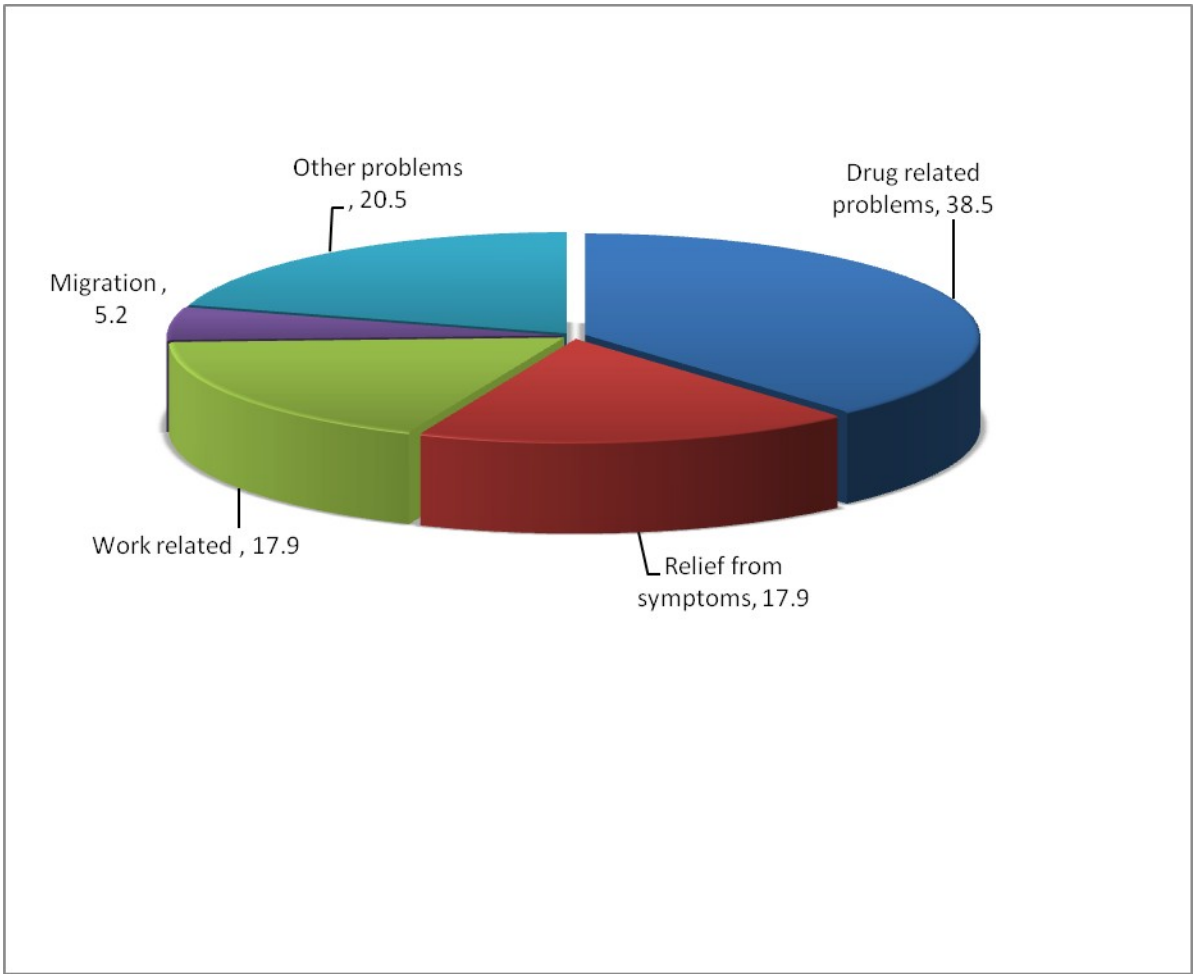
5.4 Reasons for Defaulting from Treatment

The reasons for default are enumerated in Table - 10, Fig. 10. The drug related problems like nausea, vomiting, giddiness were the leading causes for default (38.5%). The relief from symptoms and work related reasons scored equal chance for 17.9% persons to default. Migration to other distant places was a reason for default in 5.2% of cases. Domestic problems, too ill to attend, other illnesses, inconvenient DOT and dissatisfaction with treatment centers and DOT providers were the other reasons given for defaulting from treatment.

Table – 10. Percentage distribution of reasons for default.

Sl.No.	Reasons	Default	
		No.	%
1	Drug related problems	15	38.5
2	Relief from symptoms	7	17.9
3	Work related	7	17.9
4	Migration	2	5.2
5	Other problems	8	20.5
	Total	39	100

Fig. 10. Reasons for defaulting treatment among the study group.



5.5 Culture Results

The culture results of the 108 cases are posted in Table - 11.

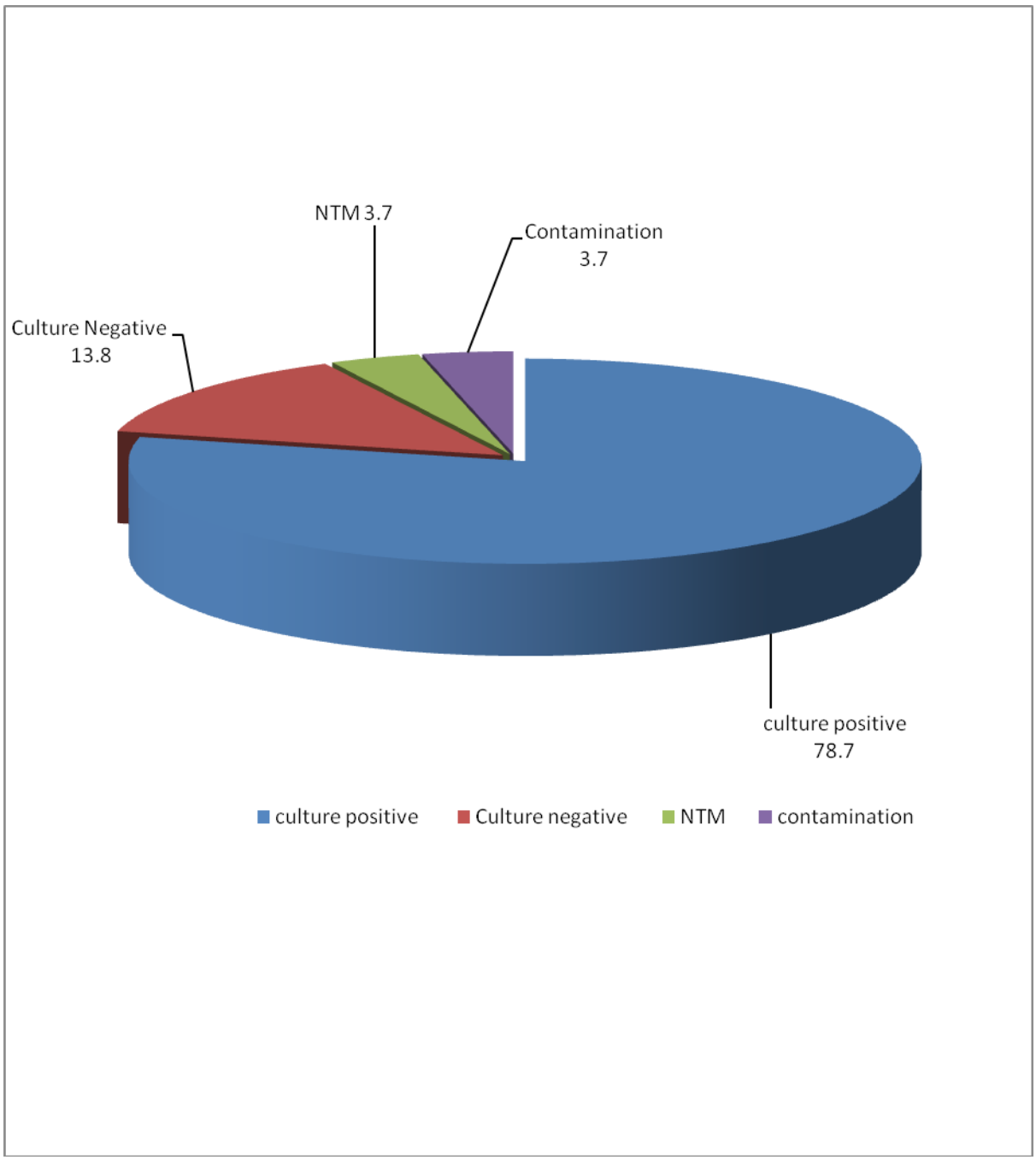
Table – 11. Culture results of the cases under study.

Sl. No.	Criteria	Total cases	Culture Positive	Others			
				Negative	Contam-ination	NTM *	Total
1	**Defaulters**	39	33	4	2	0	6
2.	**Failure**	21	14	5	1	1	7
3.	**Relapse**	27	19	5	1	2	8
4	**Category II failure**	21	19	1	0	1	2
	Total	108	85	15	4	4	23

*Non tuberculous mycobacteria

Of the 108 samples processed, 85 sputum samples (78.7%) had given a positive culture result for M.tuberculosis. 23 of the remaining samples (21.3%) had either given a negative culture or was contaminated or had grown a Non-tuberculous mycobacteria. 13.8% of the total samples processed were culture negative, 3.7% were contaminated and 3.7% had a growth of Non tuberculous mycobacteria

Fig. 11. Culture results of the cases under study.



5.5.1 Non Tuberculous Mycobacteria (NTM)

The Niacin negative cultures were identified as a growth of Non tuberculous mycobacteria. The 4 NTM cases are tabulated in Table - 12.

Table – 12. Non tuberculous mycobacteria among the study group.

Sl.No.	Study group	No. of cases	Regularity of treatment	Total duration of treatment
1.	Cat I failure	1	Regular	5 years
2.	Relapse	1	Regular	6 months
		1	Regular	6 months
3.	Cat II failure	1	Regular	11 months

All the 4 cases had taken treatment regularly as specified by the physician. 3 of them had been registered under Category II during the study period and one of the case was referred as Cat II failure.

5.6 Drug Sensitivity Results

Among the culture positive 85 cases, drug sensitivity pattern was classified and the results are posted in the Table - 13.

Table – 13. Study group wise drug susceptibility pattern.

Sl.No.	Study group	Sensitive cases		Resistant cases		Total
		No.	%	No.	%	
1.	Defaulters	21	42.0	12	34.3	33
2.	Failure	9	18.0	5	14.3	14
3.	Relapse	17	34.0	2	5.7	19
4.	Cat. II. Failure	3	6.0	16	45.7	19
	Total	50	100	35	100	85

Among the 85 culture positive cases 50 were sensitive to the first line drugs (58.8%). The remaining 35 were resistant to one or more drugs (41.2%). Among the sensitive cases 42% were defaulters, 18% were failures, 34% were relapse and the remaining 6% were category II failures. Among the drug resistant group 34.3%, 14.3%, 5.7% and 45.7% were defaulters, failure, relapse and category II failures respectively.

Of the 33 defaulters registered for treatment, 21 cases were sensitive to the first line drugs (63.6%) and 12 cases were resistant (36.4%). Among the 19 relapse cases, 17 were sensitive to the drugs (89.5%) and 2 cases were resistant (10.5%). Among the 14 Failure cases, 9 were sensitive to the drugs (64.3%) and 5 cases were resistant (35.7%). Among the 19 Category II failure cases, 3 cases

were sensitive to the drugs (15.8%) and 16 cases were resistant (84.2 %).

5.6.1 Drug Resistance

The overview of resistance to the first line anti-tuberculosis drugs among the culture positive cases is enumerated in the Table - 14.

Table – 14.

Percentage distribution of drug resistance.

Particulars	Cases	
	N	%
Total patients recruited in the survey	108	100
Total patients with DST results(n=108)	85	78.7
Total patients with susceptible isolates(n=85 henceforth)	50	58.8
Total patients with drug resistance	35	41.2
Any resistance to H	27	31.7
Any resistance to R	19	19
Any resistance to E	3	3.5
Any resistance to S	21	24.7
Total patients with mono-resistance	14	16.6
Resistance to H only	6	7.1
Resistance to R only	1	1.2
Resistance to E only	1	1.2
Resistance to S only	6	7.1
Total patients with poly resistance	21	24.7
Resistance to any 1 drug	14	16.6
Resistance to any 2 drugs	9	10.6
Resistance to any 3 drugs	10	11.8

Resistance to any 4 drugs	2	2.4
Total patients with MDR TB	18	21.2

Among the total culture positive cases cumulative drug resistance was most commonly seen to Isoniazid (27 patients 31.7%) followed by Streptomycin (21 patients, 24.7%). Resistance to Rifampicin was seen in 19 patients (22.3%) and to Ethambutol in 3 patients (3.5%).

Mono drug resistance was noted in 14 patients (16.6%). It was most commonly seen with Isoniazid and Streptomycin (6 patients each, 7.1%), followed by Rifampicin and Ethambutol (1 patient each, 1.2%).

Poly-drug resistance was observed in 21 patients (24.7%). Resistance to any two drug combination was seen in 9 patients (10.6%) and to any three drugs in 10 patients (11.8%). Resistance to all four drugs was seen in 2 patients (2.4%).

The resistance pattern for each of the drugs and the combinations is given in Table -15 and Table 16 in detail.

Table – 15.Percentage distribution of resistance for each drug.

No	**Resistant To**	**Number resistant**	**Percentage of all cases** (n=85)	**Percentage of resistant cases** (n=35)
1.	**ONE DRUG**			
	SM	6	7.1	17.1
	INH	6	7.1	17.1
	RMP	1	1.2	2.9
	EMB	1	1.2	2.9
	TOTAL	**14**	**16.6**	**40**
2.	**TWO DRUGS**			
	INH+SM	3	3.5	8.6
	INH+RMP	6	7.1	17.1

	TOTAL	**9**	**10.6**	**25.7**
	THREE DRUGS			
3	SM+INH+RMP	10	11.8	28.6
	TOTAL	**10**	**11.8**	**28.6**
4.	**ALL FOUR DRUGS**			
	SM+INH+RMP+EMB	2	2.4	5.7
	TOTAL	**2**	**2.4**	**5.7**
	GRAND TOTAL	**35**	**41.2**	**100.0**

Table – 16.Cumulative resistance to one or more anti – tuberculous drugs.

Sl.No.	Resistant to	Number Resistant	Percentage among all cases(n=85)	Percentage among resistant cases(n=35)
1.	SM	21	24.7	60.0
2.	INH	27	31.7	77.1
3.	RMP	19	22.3	54.2
4.	EMB	3	3.5	8.5
5.	SM+INH	15	17.6	42.9
6.	INH+RMP	18	21.2	51.4
7.	SM+INH+RMP	12	14.1	34.3
8.	SM+INH+RMP+EMB	2	2.3	5.7

5.6.2 Multidrug Resistant Tuberculosis

The Multi drug resistant T.B cases among the study group are tabulated in Table - 17. Among the 85 culture positive cases, 18 were MDR TB cases (21.2%).Multi-drug resistance was 6.1% among the defaulters, 14.3% among the Failures, 10.5 % among the Relapse cases and 63.2% among the Category II failure cases. Among the 18 MDR TB cases, 66.7% were Category II failure cases and the remaining cases were 11.1% in the other study groups.

Table – 17. MDR - TB in the culture positive cases.

Sl. No.	Study group	Culture positive cases	MDR. T.B		% of M.D.R. TB (n=18)
			No.	%	
1	**Defaulters**	33	2	6.1	11.1
2	**Failure**	14	2	14.3	11.1
3	**Relapse**	19	2	10.5	11.1
4	**Category II Failure**	19	12	63.2	66.7
	Total	85	18	21.2	100

5.6.3 Second Line Drug Sensitivity

Of the 18 MDR cases, 9 cases were sensitive to all the second line drugs tested. Of the 9 cases that were resistant to the drugs, resistance to Ethionamide was seen in 5 cases, to and Ofloxacin in 1 case. The Kanamycin and Ethionamide combination was resistant in 2 cases and Ethionomide plus Ofloxacin resistance was seen in 1 case. Kanamycin plus Ofloxacin resistance (XDR-TB) was not observed in the present study.

Fig.12. Drug sensitivity pattern among the study group.

5.7 HIV, TB and Drug Resistance

Among the 108 cases included under the study group, 8 cases were identified as HIV reactive at the Integrated Counselling and Testing centre, Tirunelveli medical college hospital. All the HIV reactive cases were male belonging to the rural areas. Among the 8 cases, 3 were drivers, 3 were agricultural labourers and 2 were cooly workers. The cases are enumerated in Table -18.

Table -18. Percentage distribution of defaulters, failures, relapses and Cat II Failures with HIV.

Sl.No.	Study group	Reactive		Non reactive		Significance
		No.	%	No.	%	
1	**Defaulters**	4	50.0	35	35.0	**P<0.05**
2	**Failures**	0	0	21	21.0	-
3	**Relapse**	3	37.5	24	24.0	P>0.05
4	**Cat II Failures**	1	12.5	20	20.0	P>0.05
	Total	8	100	100	100	

In comparison to the total number of cases to HIV reactivity in each of the study group, significance was observed between defaulters and HIV reactivity only and not in relapse cases and Category II failures.

The Culture results of the HIV reactive cases are given in Table -19.

Table -19.

Culture results of the HIV reactive cases.

1* - CONTAMINATED CULTURE

SL. NO.	STUDY GROUP	TOTAL CASES	HIV REAC-TIV E	% 0F HIV REACT-IVITY	CULTURE RESULT		DRUG SENSITIVITY		RESIS-TANT TO
					POS	NEG	SEN	RES	
1.	**Defaulter**	39	4	10.3	4	-	3	1	**R***
2.	**Failure**	21	0	-	-	-	-	-	**-**
3.	**Relapse**	27	3	11.1	2	1*	2	-	**-**
4.	**Cat II failure**	21	1	4.8	1	-	-	1	**S,H,R***

R- RIFAMPICIN, S- STREPTOMYCIN, I-ISONIAZID

Among the 39 defaulters included under the study, 4 cases were HIV reactive (10.3%). There

were no HIV reactive Cat I failure cases registered during the study period. Out of the 27 relapse cases, 3 patients were HIV reactive (11.1%) and among the 21 Category II failure cases, 1 patient was HIV reactive (4.8%).

One of the cultures was contaminated and drug sensitivity for the remaining 7 cases showed that 2 cases were drug resistant. One case was a defaulter and the other was a Category II failure. The HIV reactive Defaulter showed monoresistance to Rifampicin and the HIV reactive Category II failure case was resistant to Streptomycin, Isoniazid and Rifampicin.

5.8 Analysis of risk factors for Multidrug resistance

5.8.1 Influence of Regularity of Treatment on Drug Resistance

The regularity of treatment and the total courses of treatment they had undertaken prior to being registered under Category II or referred to Chennai were analysed (Table -20). Cases that had taken more than two months of treatment in each course was alone included. The drug sensitivity pattern in relation to the treatment particulars is tabulated. Of the 85 culture positive cases, 52 persons had taken single course of treatment (61.2%) and 33 persons had taken more than a single course of treatment (38.8%).

Among the 52 culture positive cases who had taken a single course of treatment 36 cases were sensitive to the drugs (69.2%) and 16 were resistant to the drugs (30.8%).

Among the 33 culture positive cases who had taken more than a single course of treatment 14 cases were sensitive to the drugs (42.4%) and 19 were resistant to the

drugs (57.6 %).

Table -20. Courses of treatment and Drug sensitivity pattern.

SL.NO.	COURSES OF TREATMENT	CULTURE POSITIVE CASES					
		SENSITIVE		RESISTANT		TOTAL	
		NO.	%	NO.	%	NO.	%
1.	**ONE**	36	69.2	16	30.8	52	100
2.	**MORE THAN ONE**	14	42.4	19	57.6	33	100
	TOTAL	50	58.8	35	41.2	85	100

The percentage of MDR cases among those who had taken treatment regularly and irregularly were analysed. (Table- 21)

Table -21. Regularity of treatment in Multi-drug resistant cases.

SL.NO.	COURSES OF TREATMENT	REGULAR			IRREGULAR			SIGNIFICANCE
		NO.	MDR	%	NO.	MDR	%	
1.	**ONE**	9	2	22.2	7	2	28.6	P>0.05
2.	**MORE THAN ONE**	1	0	0	18	16	88.8	**P<0.00**
	TOTAL	10	2	20	25	18	72.0	**P<0.001**

Among the persons who had completed the single course of treatment regularly, multi-drug resistance was observed in 22% of the cases. Among those who had irregular treatment, 29% had multi-drug resistance.

Among the persons who had taken more than single course of treatment regularly multi-drug resistance was not observed in the study group. Among those who had taken irregular treatment 89% developed multi-drug resistant tuberculosis which was statistically significant.

5.8.2 Diabetes and Drug Resistance

Table- 22. Diabetes and drug resistance pattern among the study group.

Sl. No.	Drug sensitivity pattern	Diabetics			Non-diabetics			Significance
		n	No.	%	n	No.	%	
1.	**Sensitive**	24	14	58.3	61	36	59.1	P>0.05
2.	**Resistant**	24	10	41.6	61	25	40.9	P>0.05
3.	**MDR**	10	5	50.0	25	13	52.0	P>0.05

Of the 85 culture positive cases, 24 cases were diabetic (28.2 %).Among them 10 cases were drug resistant (41.6 %) of which 5 were multi-drug resistant (50%)

61 cases were non-diabetics. (71.8 %) and among them 25 were drug resistant cases (40.9%) of which 13 cases were multi-drug resistant (52 %).No significance was observed.

5.8.3 Sex and Drug resistance

Of the 65 culture positive male cases, 27 cases were drug resistant (41.5%) and among the drug resistant cases 14 were multi-drug resistant (51.9%).

Table- 23. Sex wise distribution of Drug resistant cases.

Sl. No.	**Drug sensitivity pattern**	**Male**			**Female**			**Significance**
		n	**No.**	**%**	**n**	**No.**	**%**	
1.	**Sensitive**	65	38	58.5	20	12	60.0	P>0.05
2.	**Resistant**	65	27	41.5	20	8	40.0	P>0.05
3.	**MDR**	27	14	51.9	8	4	50.0	P>0.05

Of the 20 culture positive female, 8 cases were drug resistant (40%) and among the drug resistant cases 4 were multi-drug resistant cases (50%). No significance was observed.

5.8.4 Age and Drug resitance

Table -24. Age wise distribution of Drug Resistant cases.

Sl. No.	Drug sensitivity pattern	age ≤ 45			age> 45			Significance
		n	No.	%	n	No.	%	
1.	**Sensitive**	52	33	63.5	33	17	51.5	P>0.05
2.	**Resistant**	52	19	36.5	33	16	48.5	P>0.05
3.	**MDR**	19	11	57.9	16	7	43.8	P>0.05

Of the 85 culture positive cases, 52 cases were aged less than 45 years (61.2 %). Among them 19 cases were drug resistant (36.5 %) of which 11 were multi-drug resistant cases (57.9%).

33 cases were aged above 45 years (38.8%) and among them 16 were drug resistant cases (48.5%) of which 7 cases were multi-drug resistant (43.8 %). No significance was observed.

5.8.5 Residence and Drug resistance

Table -25. Residence wise distribution of Drug Resistant cases.

Sl. No.	Drug sensitivity pattern	Rural			Urban			Significance
		n	No.	%	n	No.	%	
1.	**Sensitive**	63	38	60.3	22	12	54.5	P>0.05
2.	**Resistant**	63	25	39.7	22	10	45.5	P>0.05
3.	**MDR**	25	12	48.0	10	6	60.0	P>0.05

Of the 85 culture positive cases, 63 cases resided in the rural areas. (74.1 %).Among them 25 cases were drug resistant (39.7 %) of which 12 were multi-drug resistant (48 %).

22 cases resided in the urban areas (25.9%) and among them 10 were drug resistant cases (45.5%) of which 6 cases were multi-drug resistant (60 %). No significance was observed.

5.8.6 Smoking habit and Drug resistance

Of the 85 culture positive cases, 65 were male cases. Among them 48 cases were smokers (53.1 %). Of them 21 were drug resistant cases (43.7%) among which 13 were multi-drug resistant (61.9%).

Table -26. Smoking habit wise distribution of Drug Resistant cases.

Sl. No.	Drug sensitivity pattern	Smoker			Non-smoker			Significance
		n	No.	%	n	No.	%	
1.	**Sensitive**	48	27	56.3	17	11	64.7	P>0.05
2.	**Resistant**	48	21	43.7	17	6	35.3	P>0.05
3.	**MDR**	21	13	61.9	6	1	16.7	**P<0.05**

17 male were non-smokers (64.7%) and among them 6 were drug resistant cases (35.3%) of which 1 case was multi-drug resistant (16.7 %). Smoking had a significant association in MDR-TB.

5.8.7 Education and Drug resistance

Table -27. Educational status wise distribution of Drug Resistant cases.

Sl.No.	Drug sensitivity pattern	Educated			Un-educated			Significance
		n	No.	%	n	No.	%	
1.	**Sensitive**	20	12	60.0	65	38	58.5	P>0.05
2.	**Resistant**	20	8	40.0	65	27	41.5	P>0.05
3.	**MDR**	8	5	62.5	27	13	48.1	P>0.05

Of the 85 culture positive cases, 20 cases were educated (23.5). Of them 8 were drug resistant (40.0%) among which 5 were multi-drug resistant (62.5%).

65 cases were uneducated (76.5%) and among them 27 were drug resistant cases (41.5%) of which 13 cases were multi-drug resistant (48.1 %). No significance was observed.

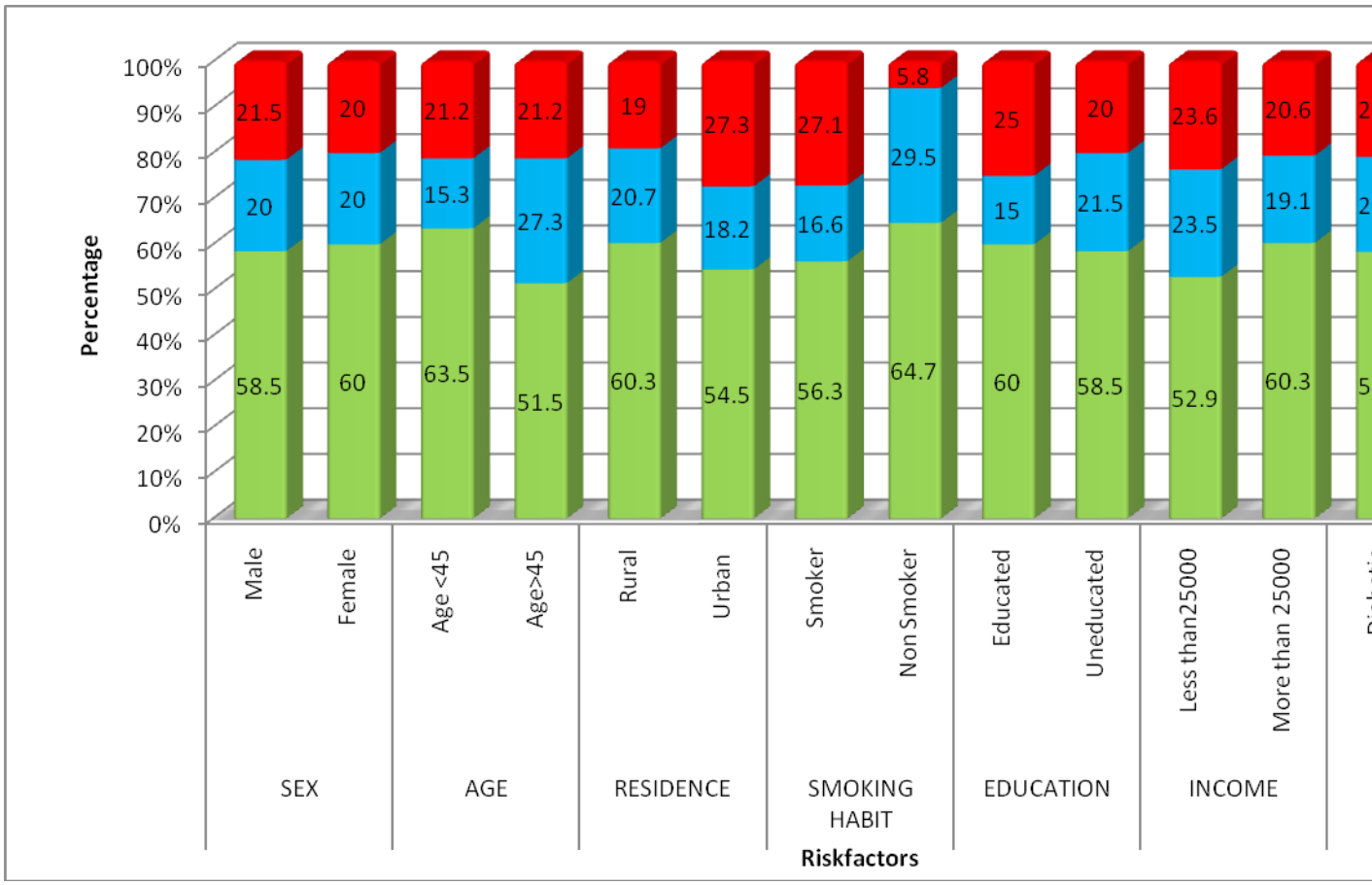
5.8.8 Income and Drug resistance

Table -28. Income wise distribution of Drug Resistant cases.

Sl. No.	Drug sensitivity pattern	More than 25000 as yearly income			Less than 25000 as yearly income			Significance
		n	No.	%	n	No.	%	
1.	**Sensitive**	17	9	52.9	68	41	60.3	P>0.05
2.	**Resistant**	17	8	47.1	68	27	39.7	P>0.05
3.	**MDR**	8	4	50.0	27	14	51.8	P>0.05

Of the 85 culture positive cases, 17 cases had an annual income of more than 25,000 rupees. (20.0 %).Among them 8 cases were drug resistant (47.1%) of which 4 were multi-drug resistant (50.0%). 68 cases had an annual income of less than 25,000 rupees. (80.0 %) and among them 27 were drug resistant cases (39.7%) of which 14 cases were multi-drug resistant (51.8%). No significance was observed.

Fig. 13. Risk factors among multi drug resistant cases



Discussion

6. DISCUSSION

A total of 108 Category II tuberculosis cases were included for this study. Since Tirunelveli Medical College Hospital is a tertiary care centre, most of the tuberculosis cases were referred from medical officers throughout the district for expert opinion before being started on Category II or being referred to Chennai. The study group comprised of cases who had failed Category I or Category III of RNTCP, defaulters and relapse cases who were currently registered for re-treatment under RNTCP-Category II and cases who had failed Category II earlier.

6.1 Age and Sex

The analysis showed that the mean age of registering for re-treatment in female cases is 37.1±12.3 and in male cases is 44.4 ±13.3. This shows that women are registered for re-treatment in the younger age than the men. Both male and female cases had notified for retreatment at middle age than the extremes of age in the present study. *Balasubramanian et al 2004* also observed that the probability of notification decreased significantly with advancing age among both the sexes.

The number of male cases in the study group was more in age group 30-59 years, in which a large proportion of men are likely to be employed. Employed men may be unable to take leave from work to attend the health care settings and would have discontinued prior treatment. The same finding that men were less likely to have successful treatment outcome was observed in a study within SAARC Countries. Better treatment compliance among women than men have been reported by *Ngamvithayapong-Yanai et al 1998* and *Balasubramanian et al 2004.* For men, being the head of the family, loss of job and fear of social isolation were reported as major reasons for discontinuation of the treatment *(Uplekar et al 2001).*

In this study, significantly more male than female cases had been registered for re-treatment. The notification of smear-positive retreatment cases was also significantly higher among men than women in a study by *Balasubramanian et al 2004* where more women than men felt inhibited

discussing their illness with family (21% vs. 14%). *Atre et al 2004* also found that women have less access to information about tuberculosis than men. In the Indian context difficulty in getting married, harassment by in-laws, dismissal from the work were reported as major barriers for women to get appropriate treatment *(Uplekar et al 1996)***.**

In the present study, men were slightly more likely to default than women but the difference was not statistically significant. In comparison to the total male and female cases included under the study, there was no significance observed in relation to sex and each of the four study groups. *Thomas et al 2004* also did not find an association between genders and relapse. The studies by *Jaggarajamma et al 2007* (24% vs. 8%)*, Balasubramanian et al* 2004 (19% vs. 8%), *Sophia Vijay et al 2003* (90% of the male defaulted) and *Santha et al 2002* (22% vs. 8%) suggest that male defaulted more than female. *Sophia Vijay et al 2002* analysed the retreatment outcome and found that gender was not significant for treatment failure but men defaulted more.

Because of the incompliance noted, gender issues are significant for development of drug resistance. But an association was not found in the present study. An European study by *Faustini et al 2006* observed more MDR cases among men. *Barroso et al 2003* and *Pande et al 2005* did not associate gender and MDR TB.

6.2 Residence

In the present study no association was observed between cases in urban and rural areas for inclusion under retreatment similar to the finding made by *Uplekar et al 1998.* There was also no significant difference in drug resistance among the rural and urban cases under study. But the study of *Deepak Almeida et al 2003* highlights an alarmingly high percentage of MDR TB in an urban area (51%) than a rural center (2%). For epidemiological reasons, there may be less onward transmission of multidrug-resistant strains in rural areas with low population densities. Residential overcrowding is a major causative factor behind the spread of TB in urban areas like Mumbai. Such overcrowding is not observed in an urban set up at Tirunelveli.

6.3 Income

Though a significant percentage of cases in each of the criteria lived below the poverty line, an association was not observed to relapse or to default or to fail from treatment in the present study. The association known for centuries between TB and poverty also applies to MDR-TB but we found no significant association between MDR-TB and family income. Poor absorption of the anti-TB medications through the gastrointestinal tract was believed to be the pathophysiologic event that resulted in sub therapeutic serum drug levels. Although several mechanisms have been proposed to explain the low serum levels of drugs in patients, the nutritional status of the patient may be a contributing factor. Poor nutritional status results in a decrease in the plasma drug concentration time curve and an increased renal clearance of unbound drug. In effect, low serum levels of anti-TB drugs result in patients being administered fewer anti-TB medications or, in some cases, even monotherapy. The latter regimen could then lead to acquired drug resistance as observed by *Byrd RP 2002.*

6.4 Education

Though the educated study groups were in less numbers for retreatment, there was no association observed in the present study. The results of *Singh et al 2002* showed that literates were better informed and more aware of the various aspects of tuberculosis as compared to the illiterates.

Few authors worldwide had the concern of investigating the patient's educational level as a risk factor for MDR-TB. *Al Jarad et al 1994, London* and *Murray et al 2000, South Africa* found no association with MDR-TB. Two Brazilian studies by *Natal et al 2000* and *Barroso et al 2003* revealed an association between MDR-TB and lack of school education.

6.5 Smoking Habit

It was found that smoking was a significant risk factor among relapse cases (33% vs. 9%), Category I (23% vs. 5%) and Category II failures (22 % vs. 14%) in the present study.

Studies by *Joanna d'Arc et al 2008* (Odds ratio 2.5), *Thomas et al 2005* (18.1% vs. 7.3%) *and*

Kolappan et al 2003 (Odds ratio 2.5) support the observation that smokers are significantly more likely to relapse than nonsmokers. *Santha et al 2000*, observed an association between smoking and treatment failure (Odds ratio 8.4). A study by *Subodh Katiyar et al 2008* observed that 47% of the smokers had failed Category II treatment.

Although the number of smokers were high among defaulters (22% vs. 30%) in the study group, the difference was not statistically significant in the present study. *Santha et al 2000* (23% vs. 13%) also found no association despite the high percentage of smokers. However *Chandrasekaran et al 2005* (14.6% vs. 5.9%) showed that smoking was a risk factor to default from treatment.

There was a significant association between smoking and multidrug resistant tuberculosis in the present study (62% vs. 17%). *Barroso et al 2003* (60 % vs. 40 %) identified that smoking was associated with MDR-TB in their analysis. *Ruddy et al 2005* identified smoking as a risk factor for Isoniazid resistance. However *Pande et al 2005,* India observed that smoking had no relation to infection with MDR organism.

6.6 Diabetes

There was no evidence for an increased risk of retreatment and MDR TB among people with diabetes in the present study. A systematic review of 13 observational studies on the relation between diabetes and tuberculosis by *Christie et al 2008* found consistent evidence for an increased risk of TB among people with diabetes. *Patel et al 1989* and *Ezung et al 2002* reported that tuberculosis was found to be the most common complicating illness in patients with diabetes mellitus.

Mona Bashar et al 2001 in his study found out that diabetic patients were more than five times as likely to have infection with a multi drug resistant strain of tuberculosis. But drug resistance to first line anti-TB drugs was not found to be associated with diabetes mellitus in studies of *Rupak Singla et al 2003*(0%) and *Barroso et al 2003*(8%). In a study by *Subhash et al 2003* CMC, Vellore on patients with diabetes and Tuberculosis, only 26% of the diabetic subjects with tuberculosis had MDR TB,

which ruled out an association between both.

6.7 Regularity of Treatment

The percentages of patients on irregular treatment were statistically significant among defaulters (45% vs. 23%) and Category II failure case (26% vs. 9%) in this study. There was no significance observed among relapse cases and irregular treatment.

Sophia Vijay et al 2003 (80%)*, Santha et al 2000*(Odds ratio 3.9%) observed that higher default rates were associated with irregular treatment as in the present study. However *Thomas et al 2005* found that the patients who were irregular on treatment were twice as likely to relapse as those who were regular (20 % vs. 9%).

There is virtually a consensus among researchers regarding the fact that the number of previous treatments is a risk factor for MDR TB and our study confirmed this association. *Pande et al 2005* observed the prevalence of MDR-TB in patients with past history of ATT at two centres in Delhi to be 44.7% and 20% which was statistically significant in their study. 84.8% of MDR TB cases had irregular and interrupted treatment as reported by *Vasanthakumari et al 1997.* Poor past compliance to treatment (Odds ratio 6.6) was associated with MDR TB in the study of *Sharma et al 2003.*

Analysis of the abandoned and irregular treatments showed a higher risk of developing MDR TB in studies of *Espinal et al 2001* and *Central TB Division report 2006. Davies et al 1998* in Bombay proved the incidence to be over 50% in those who had previous irregular treatment. *Barroso et al 2003* observed that number of previous treatments (Odds Ratio 4.58) and irregular treatments (Odds ratio 5.14) were significant risk factors for MDR TB**.** *Faustini et al 2006* observed that previous treatment was the strongest determinant of MDR-TB in Europe.

A study by *Costello et al 1980* found that 41% patients with previous treatment for TB developed drug-resistant TB, and the percentage increased with increasing duration of the previous treatment.

Previous anti-tuberculosis chemoprophylaxis (Odds ratio 4.8) by *Vargas et al 2006* and previous TB-treatments by *Clendenes et al 2002* were observed as significant risk factors for developing MDR TB in HIV patients.

6.8 Reasons for Defaulting from Treatment

Reasons for default from treatment like drug related problems, relief from symptoms, work related problems, treatment from other private or public health facility, domestic problems, stigma, too ill to attend, old age, other illnesses, inconvenient DOT and dissatisfaction with treatment centre and DOT provider as stated by the study group were also observed in studies of *Jaggarajamma et al 2007, Pronab Chatterjee et al 2003* and *Sophia Vijay et al 2003*. The other reasons like distance from treatment centre, lack of motivation and difficulty in DOTS timing as observed in other studies were not stated as reasons to default because the DOT provider was accessible to the patient any time. However studies of *Jaggarajamma et al 2006, Sophia Vijay et al 2003* and *Sudha Ganapathy et al 1994* have brought out migration as an important factor for treatment default, which was observed only in 5% of the defaulters in the present study.

6.9 Culture Results

Of the 108 samples processed, 85 (78.7%) showed a positive growth for M. tuberculosis. 13.9% of the total samples processed were culture negative, 3.7% were contaminated and 3.7% had a growth of Non Tuberculous Mycobacteria.

A negative culture result with the specimen containing tubercle bacilli may be due to several reasons. In patients receiving treatment, the organisms may have lost their ability to grow on a culture media and be practically dead. Patients being treated with a Rifampicin containing regimen often become culture negative by about the third week of treatment, although they may be still smear positive, the bacilli are dead or nonviable. The sputum specimens exposed to heat, stored too long, dried out or contaminated also yield a negative culture. Excessive decontamination procedures before

inoculation, over heating before centrifugation, inadequate culture media and deficient incubation also result in a negative culture. Positive smears may be caused by non- tuberculous mycobacteria (*Toman's Tuberculosis p.44-45).*

6.9.1 Non Tuberculous Mycobacteria

If only a few colonies of non-tuberculous mycobacteria (NTM – often pigmented, with smooth morphology or PNB positive) were grown, it was taken as culture negative. More than 20 colonies of only NTM in both slopes were present in 4 cases in the study group. The pathogenic role of NTM could not be established in this study, since repeat isolation was not possible. The isolation rate was 3.7%. The isolation rate of NTM from India has been reported ranging from 0.5 percent to 8.6 percent in several studies by *Sivasankaril et al 2006, Paramasivan et al 1985 &1986, Hardas et al 1984* and *Pathak et al 1973*. The reports of *Karak et al* from Kolkotta, shows an NTM prevalence of 17.4% from sputum specimens, much higher than the above studies.

Although none of the patients were HIV positive, isolation of these NTM strains show that these strains are re-emerging as potential pathogens. Regular documentation and reporting of these NTMs from clinical settings along with their sensitivity profiles is essential to be aware of the possible spectrum of diseases associated and preferred treatment options.

6.9.2 Drug Sensitivity

The study group patients claimed to have taken anti-tuberculosis therapy without improvement; however, 50 (58.8%) isolates were sensitive to all four first-line drugs (INH, RIF, SM and EMB) that were tested. Resistance observed in this study was 41.2%.

The percentage of sensitivity among defaulters, Category I failures, relapse and Category II failures was 63.6%, 64.3%, 89.5% and 15.8% respectively, showing that significant proportion of the

cases were sensitive to the drugs. The percentage of resistance among defaulters, Category I failures, relapse and Category II failures was 36.4%, 35.7%, 10.5% and 84.2 % respectively.

Default and failure patients had high drug resistance than the relapse cases. Except for the Category II failures in whom a high degree of resistance and low level of sensitivity was observed, all the other categories showed a significant level of drug sensitivity. This study shows that all re-treatment cases can be adequately treated with the category II regimen and referring the Category II failures for drug susceptibility testing and further management to referral centres may be adequate. The low success rate to the CAT-II regimen was mainly due to the high default during treatment. If treatment compliance can be ensured for all patients registered to CAT-II regimen we can have a successful treatment outcome. *Pauline Joseph et al 2006* also observed the same findings in their study.

6.9.3 Drug Resistance

Among all the culture positive patients (n=85) resistance to one drug was noted in 14 patients (16.6%), to two drugs in 9 patients (10.6%), to three drugs in 10 patients (11.8 %) and to all four drugs in 2 patients (2.4%). The similar pattern of resistance was observed by *Shah et al, 2002* and *Sophia Vijay et al 2002* except for all four drugs which was 15.21% in the former and 4% in the latter.

Single drug resistance was most commonly seen with Isoniazid and Streptomycin (6 patients each, 7.1%), followed by Rifampicin and Ethambutol (1 patient each, 1.2%).

The present study shows high degree of resistance to Streptomycin similar to study of *Sophia Vijay et al 2002* which showed resistance to Streptomycin in 9.3%, followed by Isoniazid in 8.4%, Rifampicin in 1.8% and Ethambutol in 0 cases. This is in contrast to study of *Shah et al 2002*, which showed less resistance to Streptomycin, where the pattern of resistance was Isoniazid in 7.5%, followed by Streptomycin in 1.4%, Rifampicin in 0.97% and Ethambutol in 0.4%.

Resistance to Isoniazid plus Rifampicin alone was seen in 6 patients (7.1%) similar to study of *Shah et al 2002* which showed 9.2 % but 4.9% by *Sophia Vijay et al 2002*.

Most of the Rifampicin resistant cases were also resistant to Isoniazid. This has also been

observed in studies of *Trivedi et al 1988* and *Shah et al 2002*.

When comparing resistance to individual drugs among the total drug-resistant cases (35 patients, 41.2%), cumulative resistance to Isoniazid was highest (31.7%), followed by Streptomycin (24.7%), Rifampicin (22.3%) and Ethambutol (3.5%). Resistance to Isoniazid was also found to be high in several studies. It was 15% in Bombay *(Chowgule et al 1998)*, 27.4% in Bangalore *(Sophia Vijay et al 2002),* 41% in Chennai *(Deivanayagam et al 2002),* 57.18% in Gujarat *(Shah et al 2002),* 61.76% in Jodhpur (*Mathur et al 2000)* and 81% in North-Arcot (*Paramasivan et al 2002).*

Since resistance to Streptomycin was high in the present study, it was the second commonest drug for which resistance was observed unlike the above studies except 23% shown by *Sophia Vijay et al 2002*.

All the above studies had Rifampicin resistance in the second place. Drug resistance to Rifampicin in the present study was 22.3% which is similar to that reported from Jaipur 28.2% by *Malhotra et al 2002*, New Delhi 33.7% by *Jain et al 1992,* Gujarat 37.3% by *Trivedi et al 1988* and Gujarat 37.47% by *Shah et al 2002* though Bombay reports a very high incidence of Rifampicin resistance of 66.8% *(Chowgule et al 1998).*

Ethambutol was the least resistant drug in all the studies as in the present study, although the percentage was very less (3.5%) compared to 6.6% in Bangalore *(Sophia Vijay et al 2002),* Bombay 8.4% *(Chowgule et al 1998),* Indore 22% *(Hemvani et al 2001),* Chennai 28.7% (*Deivanayagam et al 2002),* Gujarat 35.45% (*Shah et al 2002)* and Jodhpur 39.39% (*Mathur et al 2000).*

6.9.4 Multidrug Resistant Tuberculosis (MDR-TB)

A high degree (21.2 %) of MDR-TB was observed among the study group. This was in accordance to most of the studies in India. Proportion of MDR- TB in re-treatment cases varied from 100% Raichur, Karnataka, 69% North-Arcot study, *(Paramasivan CN et al 2002),* Jodhpur 38.2% *(Mathur et al 2000),* Jaipur 24.3 % *(Malhotra et al 2002),* 20.3 % *(Paramasivan, 1998)*, 17.2%-Gujarat *(Anti-tuberculosis drug resistance in the world, WHO Fourth Global Report 2008),* Gujarat 15.77 %

(Shah et al 2002), Gujarat 14 % *(Dam et al 2005),* Bangalore 12.8% *(Sophia Vijay et al, 2002*), Indore 8.1%. *(Hemvani et al 2001).*

There is concern regarding the efficacy of CAT-II regimen for re-treatment of TB patients especially for 'Failure' cases, since a high proportion of them may be having MDR-TB. In the present study, the percentage of MDR-TB in defaulters, Relapse and Category I failures was 11.1 % each and it was higher (66.7%) among the Category II failure cases. This study like that of *Santha et al 2005* and *Pauline Joseph et al 2006* shows that the RNTCP policy in India of treating all retreatment cases with the WHO recommended re-treatment regimen may be adequate except for the MDR-TB patients. DST should be done for patients who remain sputum smear positive during the retreatment period and appropriate regimens should be started as early as possible for better treatment outcome and to reduce transmission of drug resistant TB.

6.9.5 Extensively Drug-Resistant Tuberculosis (XDR-TB)

Of the 18 MDR cases, 9 cases were sensitive to all the second line drugs tested. XDR-TB cases were not seen in the present study. Of the 9 cases that were resistant to the drugs, the resistance to Ethionamide was seen in 8 cases explaining cross-resistance of Ethionamide with INH. However, the fact that all tested isolates were resistant to isoniazid but one was not resistant to ETH might suggest that the mutations leading to drug resistance are located in different regions of the genome. Kanamycin resistance was seen in 2 cases, both of which had resistance to Streptomycin also. There is no cross resistance between Ofloxacin and other antituberculosis drugs but Flouroquinolones are the widely prescribed antibiotics for all infections explaining the emergence of drug resistance.

Rajesh Mondal et al 2007, reported 7.4 % of XDR TB cases, the first ever report from India. A limitation to accurate detection of XDR TB is because; the existing tests for resistance to second line drugs is not yet standardized and are less reproducible than results for first line drugs. Only then second-line treatment can be individualized, based on in-vitro drug resistance, or can be standardized.

6.10 HIV, TB and Drug Resistance

Diagnosis of TB in HIV infected patients is difficult because of absence of fever and symptoms, negative sputum smears, atypical chest radiographs, higher prevalence of EPTB especially at inaccessible sites and resemblance to other opportunistic pulmonary infections *(Sharma et al 2004).* Since smear positive cases were alone included in the present study, only 8 cases were found to be HIV reactive. More defaulters had HIV reactivity than any other group in the present study. *Connolly et al 1999, South Africa* reports that being diagnosed for HIV was significantly associated with default. But *Epco Hasker et al 2008, Tashkent* finds no apparent association with default.

A review of the published literature by *Ormerod LP 2005* and *Sharma et al 2004*, suggests that, in the early 1990s, several institutional outbreaks of MDR-TB among HIV-infected patients drew attention to the problem. In this study, HIV and MDR co-infection was seen in only one case and

Rifampicin monoresistance was observed in a case. Since the HIV cases were not in significant numbers, an attribute could not be made out. Studies of *Barroso et al 2003, Spellman et al 1998, Asch et al 1996* have found that MDR-TB is not more common among people infected with HIV. But *Swaminathan et al 2005* in their study have observed MDR-TB in 13.5 % of the retreatment HIV cases. *Sharma et al 2003* and *Jasmin Johnson et al 2003* have demonstrated Rifampicin monoresistance in HIV patients.

Summary

7. SUMMARY

The present study aimed at finding the Drug susceptibility profile among previously treated pulmonary tuberculosis cases, analyse the risk factors that had led to the retreatment of the cases and identify the risk factors of MDR-TB

108 cases of retreatment pulmonary tuberculosis were included for the study for whom the risk factors for inclusion under Category II as defaulters, treatment failures or relapse cases were analysed. Specimens were cultured at Tirunelveli medical college. For the positive cultures, drug sensitivity testing was done at Tuberculosis Research Centre (ICMR), Chetput, Chennai. The risk factors for acquiring MDR TB were analysed.

- Females were registered for re-treatment in the younger age (37.1±12.3) than males (44.4 ±13.3) and middle aged persons were in high numbers in both the sexes.
- Males were highest in the age group 30-59 years, where most of them were employed and discontinued prior treatment.
- More males (79.6%) were registered for retreatment than females (20.4%) suggesting that while men were better able to access TB treatment from a DOT facility, the need to earn a livelihood acts as a barrier to complete treatment for them.
- Women, on the other hand, have greater difficulty reaching an appropriate facility, but those who do, usually complete treatment.
- Area of residence, family income, education, gender were not risk factors for retreatment or MDR TB.
- Smoking was a significant risk factor among Relapse and Failure cases. It was also a significant risk factor to acquire MDR TB.
- Diabetes was not a risk factor to default or relapse or fail from treatment and was also not a risk

factor to acquire MDR TB.

- Irregular and interrupted treatment was a risk factor among Defaulters and Failures and it also consistently led to the development of MDR TB.
- Drug related problems like nausea, vomiting, giddiness (38.5%) were the leading cause for default.
- 8 cases were HIV reactive in the total study group. HIV cases were more among defaulters.
- HIV reactive defaulter showed monoresistance to Rifampicin. HIV reactive Category II failure case was multidrug resistant.
- Of the 108 samples processed, 78.7% showed a positive growth for M. tuberculosis, 13.9% were culture negative, 3.7% were contaminated and 3.7% had a growth of Non tuberculous mycobacteria. Since repeat isolation was not possible for the NTM, its pathogenic role could not be established in this study. The patients have been registered for retreatment unnecessarily.
- Among the 85 culture positive cases, 50 (58.8%) were sensitive to the first line drugs and 35 (41.2%) were resistant to one or more drugs.
- Among the 50 sensitive cases 42% were defaulters, 18% were failures, 34% were relapse and 6% were category II failures.
- Among the 35 drug-resistant cases 34.3% were defaulters, 14.3% were failures, 5.7% were relapse, and 45.7% were category II failures.
- Of the 33 defaulters, 21 (63.6%) were sensitive and 12 (36.4%) were resistant.
- Of the 14 failure cases, 9 (64.3%) were sensitive and 5 (35.7%) were resistant.
- Of the 19 relapse cases, 17 (89.5%) were sensitive and 2 (10.5%) were resistant.
- Of the 19 Category II failure cases, 3 (15.8%) were sensitive and 16 (84.2 %) were resistant.
- Resistance to one drug was noted in 14 (16.6%), to two drugs in 9 (10.6%), to three drugs in 10 (11.8 %) and to all four drugs in 2 (2.4%) cases.
- Monodrug resistance was most commonly seen with Isoniazid and Streptomycin (6 patients

each, 7.1%), followed by Rifampicin and Ethambutol (1 patient each, 1.2%).

- Among the 35 drug resistant cases, cumulative resistance was highest to Isoniazid (27 patients 31.7%) followed by Streptomycin (21 patients, 24.7%), Rifampicin (19 patients, 22.3%) and Ethambutol (3 patients, 3.5%).
- 18 were MDR TB (21.2%) among the culture positive cases. Among the 18 MDR TB cases, 66.7% were Category II failure cases and 11.1% in the each of the other study groups.
- No XDR TB cases were present. The treatment of MDR-TB becomes difficult since Second Line Drugs must be used, which are less potent and not as well tolerated as first-line agents. Susceptibility testing of M.tuberculosis to SLDs is difficult, expensive, and not well standardized.

Conclusion

8. CONCLUSION

- Age, sex, residence, education, income, diabetes were not risk factors for retreatment tuberculosis and multidrug resistant tuberculosis.
- Smoking was a significant risk factor among relapse, failure and MDR cases. There is a need to devise effective strategies for counselling patients about the impact of smoking on their cure.
- Irregular and interrupted treatment was a risk factor among defaulters, failures and multidrug resistant cases. Ensuring adherence to a full course of treatment is the key to cure TB patients and prevent the emergence of drug resistance.
- Drug related problems like nausea, vomiting, giddiness were the leading cause to default from treatment. The innovative strategies in health education are the need of the hour.
- HIV reactivity was noted among defaulters in the present study and not among MDR cases.
- As drug sensitivity testing is not routinely performed, NTM cases had been registered for retreatment unnecessarily.
- Though registered for retreatment, most of the isolates were sensitive to all the first line drugs and hence can be successfully treated with a category II regimen if they are compliant enough.
- Drug resistance was more among prior treatment failure cases, necessitating the need for timely culture and sensitivity testing for those who remain sputum positive during the course of treatment to curb the spread of multidrug resistant strains.

Bibliography

9. BIBLIOGRAPHY

Al Jarad N, Parastatides S, Paul EA, Sheldon CD, Gaya H, Rudd RM, et al. Characteristics of patients with drug resistant and drug sensitive tuberculosis in East London between 1984 and 1992. *Thorax* 1994; 49:808-10.

Anti-Tuberculous drug resistance in the world, *Fourth Global Report 2008, The WHO/IUATLD Global Project on Anti-tuberculosis Drug Resistance Surveillance 2002-2007.*

Asch S, Knowles L, Rai A, et al. Relationship of Isoniazid resistance to human immunodeficiency virus infection in patients with tuberculosis. *Am J Respir Crit Care Med* 1996; 153:1708–1710.

Ashry Gad, et al, Compliance with antituberculosis drugs among tuberculosis patients in Alexandria, Egypt; Volume 3, Issue 2, 1997, Page 244-250. http://www.emro.who.int/ Publications/EMHJ/0302/06.htm.

Atre SR, Kudale AM, Morankar SN, Rangan SG, Weiss MG. Cultural concepts of tuberculosis and gender among the general population without tuberculosis in rural Maharashtra, India. *Tropical Med Int Health*. 2004;9(11):1228-38.

Augustynowicz-Kopec E, Zwolska Z, Jaworski A, Kostrzewa E, Klatt M. *Int J Tuberc Lung Dis.* 2003 Jul; 7(7):645-51. Drug-resistant tuberculosis in Poland in 2000: second national survey and comparison with the 1997 survey.

Balasubramanian V.N., Oommen K., and Samuel R. DOT or not? Direct observation of anti-tuberculosis treatment and patient outcome, Kerala State, India. *Int J Tuberc Lung Dis* 2000,4(5):409-413.

Balasubramanian, R. Garg, T. Santha, P. G. Gopi, R. Subramani, V. Chandrasekaran,A. Thomas, R. Rajeswari, S. Anandakrishnan, M. Perumal, C. Niruparani, G. Sudha,K. Jaggarajamma, T. R. Frieden, P. R. Narayanan. Gender disparities in tuberculosis: report from a rural DOTS programme in south India. *Int J Tuberc Lung Dis* 8(3):323–332,2004.

Barnhoorn F, Adriaanse H. In search of factors responsible for noncompliance among tuberculosis patients in Wardha District, India. *Social Science and Medicine* 1992, 34(3):291- 306.

Barroso Elizabeth Clara, Rosa Maria Salani Mota, Raimunda Oliveira Santos, Ana Lúcia Oliveira Sousa, Joana Brasileiro Barroso, Jorge Luís Nobre Rodrigues, Risk factors for acquired multidrug-resistant tuberculosis, *J Pneumol* 2003;29(2):89-97.

Boelaert J R, Gomes M S, Gordeuk V R. Smoking, iron and TB. *Lancet* 2003; 362: 1243–1244.

Borgdorff Martien W., Floyd Katherine, Broekmans Jaap F. Interventions to reduce tuberculosis mortality and transmission in low- and middle-income countries. *Bull of WHO* vol 80:pg 217-227: 2002.

Byrd RP. Malnutrition and pulmonary tuberculosis. *Clin Infect Dis*. 2002;35: 633-4.

Canetti G, Grosset J, Perccentage of INH resistant and SM resistant variants in wild strains of M. tuberculosis on LJ medium. *Annales de l' Institut Pasteur*, 1961, 101: 28-46.

Carpenter JL, Obnibene AJ, Gorby EW, Neimes RE, Koch JR, Perkins WL. Antituberculosis drug resistance in south Texas. *Am Rev Respir Dis* 1983; 128: 1055-8.

CDC Outbreak of multidrug-resistant tuberculosis at a hospital - New York City, 1991. *MMWR Morb Mortal Wkly Rep* 1993; 42: 427, 433-4.

CDC Transmission of multidrug-resistant tuberculosis among immunocompromised persons in a correctional system, New York, 1991. *MMWR Morb Mortal Wkly Rep* 1992; 41: 507-9.

Central TB Division Report , Directorate General of Health Services, Ministry of Health & Family Welfare, Government of India. Revised national tuberculosis control programme: *DOTSPlus guidelines 2006.*

Chakrabarthi A, Sharma, Dubey M.L Isolation rates of different mycobacterial species from Chandigarh. *Indian J Med Res* 1990; 91:111-4.

Chandrasekaran.V, P.G.Gopi, R.Subramani, A.Thomas, K.Jaggarajamma, P.R.Narayanan. Default During The Intensive Phase Of Treatment Under DOTS Programme, *Indian J Tuberc* 2005; 52:197-202.

Chowgule RV, Deodhar L. Pattern of secondary acquired drug reistance to antituberculosis drug in Mumbai, India -1991-1995. *Indian J Chest Dis Allied Sci* 1998;40:23-31.

Christie Y. Jeon, Megan B. Murray, Diabetes Mellitus Increases the Risk of Active Tuberculosis: A Systematic Review of 13 Observational Studies PLoS Med 5(7): e152. doi:10.1371/journal.pmed.0050152 July 15, 2008.

Clendenes M, Ticona E, Jave O, Salazar F; International Conference on AIDS. Risk factors for multidrug-resistant-Tuberculosis in HIV patients in Lima - Peru. Int Conf AIDS. 2002 Jul 7-12; 14: abstract no. ThPeC7554.

Coninx R, Mathieu C, Debacker M, Mirzoev F, Ismaelov A, de Haller R, et al. First-line tuberculosis therapy and drug-resistant Mycobacterium tuberculosis in prisons. *Lancet* 1999; 353 : 969-73.

Connolly, GR Davies, D Wilkinson. Who fails to complete tuberculosis treatment? Temporal trends and risk factors for treatment interruption in a community based directly observed therapy programme in a rural district of South Africa. *Int J Tuberc Lung Dis* 1999; 3(12): 1081-1087.

Costello HD, Caras GJ, Snider DE, Jr. Drug resistance among previously treated tuberculosis patients: a brief report. *Am Rev Respir Dis* 1980; 121:313-16.

Crofton J. and Mitchison D.A. (1948) Streptomycin resistance in pulmonary tuberculosis. *Br Med J.* 2: 1009.

Crofton J. The assessment and treatment of drug-resistance problems in tuberculosis. *Journal of the Irish Medical Association* 1970; 63:75-8.

Crofton J, Chaulet P, Maher D, Grosset J, Harris W, Horne N. Guidelines for the management of drug resistant tuberculosis. WHO/TB/96.210 Rev1. Geneva: *World Health Organization;* 1997.

Dam.T, M. Isa and M. Bose, Drug sensitivity profile of clinical Mycobacterium tuberculosis isolates – a retrospective study from a chest disease institute in India. *Journal of Medical Microbiology*, 2005; 54, 269–271.

Das BK, Sharma VK, Bhanu LN, Saxena SN and Bhardwaj BK.Characterization of mycobacterial strains from clinical specimens. *Indian J Pathol Micobiol* 1982; 25. 74.

Datta M, Radhamani MP, Selvaraj R, Paramasivan CN, Gopalan BN, Sudeendra CR, et al. Critical assessment of smear-positive pulmonary tuberculosis patients after chemotherapy under the district tuberculosis programme. *Int J Tuberc Lung Dis* 1993; 74: 180-6.

Davies PDO, Udwadia ZF, Hakimizon A, Rodrigues C, Jillisgon T, Mehta A. Drug resistant tuberculosis in Mumbai,

India. *Thorax* 1998; 53 :(suppl 4): A32.

De Rossi E, Branzoni M, Cantoni R, Milano A, Riccardi G, Ciferri O. A Mycobacterium tuberculosis gene conferring resistance to small cationic dyes and inhibitors. *J Bacteriol* 1998; 180 : 6068-71.

Deepak Almeida, Camilla Rodrigues, Zarir F Udwadia, Ajit Lalvani, G D Gothi, Pravin Mehta, Ajita Mehta, Incidence of multidrug-resistant tuberculosis in urban and rural India and implications for prevention. *Clin Infect Dis*. 2003 Jun 15; 36 (12):52-4.

Deivanayagam.C.N., S.Rajasekhar, R.venkatesan, A. Mahilmaran,P.R. Khaiser Ahamed, S. Annadurai,N. Ravichandran,R.Pencilliah, Prevalence of Scquiredv MDR TB &HIV coinfection. *Indian J Chest Allied Science,* 2002; 44: 237-242.

Domingo Palmero, Viviana Ritacco, Martha Ambroggi, Marcela Natiello, Lucía Barrera, Lilian Capone, Alicia Dambrosi, Martha Di Lonardo, Nélida Isola, Susana Poggi, Marisa Vescovo, Eduardo Abbate, Multidrug-Resistant Tuberculosis in HIV-Negative Patients, Buenos Aires, Argentina August 2003 issue of *Emerging Infectious Diseases.*

Drake, J. W. 1999. The distribution of rates of spontaneous mutation over viruses, prokaryotes, and eukaryotes. Ann. N. Y. Acad. *Science.*870:100–107.

Duvall CW. Studies in atypical forms of tubercle bacilli isolated directly from the human tissues in cases of primary cervical adenitis. *J Exp Med* 1908; 9: 403-29.

East African/British Medical Research Councils. 1981. Controlled trial of five short course regimens of chemotherapy regimens for pulmonary tuberculosis. *Am. Rev. Respir. Dis*. 123:165–170.

Epco Hasker, Maksad Khodjikhanov, Shakhnoz Usarova,Umid Asamidinov, Umida Yuldashova, Marieke J van der Werf, Gulnoz Uzakova and Jaap Veen Default from tuberculosis treatment in Tashkent, Uzbekistan; Who are these defaulters and why do they default? *BMC Infectious Diseases* 2008, 8:97.

Espinal MA, Kim SJ, Suarez PG, Kam KM, Khomenko AG, Migliori GB, et al. Standard short-course chemotherapy for drug-resistant tuberculosis: treatment outcomes in 6 countries. *JAMA* 2000; 283: 2537-45.

Espinal MA, Laserson K, Camacho M, et al. Determinants of drug-resistant tuberculosis: analysis of 11 countries. *Int J Tuberc Lung Dis* 2001; 5:887–893.

Ezung T, Devi NT, Singh NT, Singh TB. Pulmonary tuberculosis and diabetes mellitus-a study. J. of *Indian Medical Association*. 2002, 100, 376.

Faustini A, A J Hall, C A Perucci Risk factors for multidrug resistant tuberculosis in Europe: a systematic review *Thorax* 2006;61:158–163.

Ferreira RM, Saad MH, Silva MG, Fonseca S., Nontuberculous mycobacteria : one year clinical isolates identification in Tertiary Hospital Aids Reference Center, Brazil, in pre highly active antiretroviral therapy era. *Mem Inst Oswaldo Cruz*. 2002; 97:725-9.

Fischl MA, Daikos GL, Uttamchandani RB, Poblete RB, Moreno JN, Reyes RR, et al. Clinical presentation and outcome of patients with HIV infection and tuberculosis caused by multiple-drug-resistant bacilli. *Ann Intern Med* 1992; 117: 184-90.

Gollapudi S, Reddy M, Gangadharam P, Tsuruo T, Gupta S. Mycobacterium tuberculosis induces expression of Pglycoprotein in promonocytic U1 cells chronically infected with HIV type 1. *Biochem Biophys Res Commun* 1994; 199 : 1181-7.

Gordin FM, Nelson ET, Matts JP, Cohn DL, Ernst J, Benator D, et al. The impact of human immunodeficiency virus infection on drug-resistant tuberculosis. *Am J Respir Crit Care Med* 1996; 154 : 1478-83.

Grumbach, F., G. Canetti, and M. Le Lirzin. 1970. Durable character of the sterilization of experimental tuberculosis in mice by rifampicin-isoniazid association: cortisone test. Rev. Tuberc. *Pneumol.* 34:312–319.

Hardas UD, Jayaram VS. Differential identification of mycobacteria. *Indian J Tub* 1984; 31: 11.

Hemvani, DS Chitnis, GC Bhatia, N Sharma. Drug resistance among tubercle bacilli from Pulmonary Tuberculosis cases in Central India. *Indian Journal of medical sciences* 2001, Volume 55, Issue 7, Page: 382-92.

Hobby, G. L., and T. F. Lenert. 1952. Resistance to isonicotinic acid. Am. Rev. Tuberculosis 65:77.

Iseman M.D. and Madsen L.A. (1989) Drug-resistant tuberculosis. *Clin Chest Med*. 10: 341-353.

Jaggarajamma.K, G. Sudha, V. Chandrasekaran, C. Nirupa, A. Thomas, T. Santha, M. Muniyandi and P.R. Narayanan. Influence of drug susceptibility on treatment outcome and susceptibility profile of 'failures' to CATEGORY II regimen, *Indian J Tuberc* 2006; 53:141-148.

Jaggarajamma.K, G. Sudha, V. Chandrasekaran, C. Nirupa, A. Thomas, T. Santha, M. Muniyandi and P.R. Narayanan.Reasons for non-compliance among patients treated under revised national Tuberculosis control programme (RNTCP), Tiruvallur district, South India, *Indian J Tuberc 2007*; 54:130-135.

Jain NK, Chopra KK, Prasad G. Initial and acquired INH and rifampicin resistant to Mycobacterium tuberculosis and its implication for treatment. *Indian J Tub* 1992; 39:180- 186.

Jasmin Johnson,Anju Kagal,Renu Bharadwaj. Factors associated with drug resistance in pulmonary Tuberculosis. *Indian J Chest Dis Allied Science,* 2003; 45:105-109.

Jesudason MV,P Gladstone, Non Tuberculous mycobacteria isolated from clinical specimens at a tertiary care hospital in South India . *Indian journal of medical microbiology*, 2005: 23 (3):172-175.

Joanna d'Arc Lyra Batista, Maria de Fátima Pessoa Militão de Albuquerque, Ricardo Arraes de Alencar Ximenes, and Laura Cunha Rodrigues, Smoking increases the risk of relapse after successful tuberculosis treatment *Int J Epidemiol.* 2008 August; 37(4): 841–851.

Johnson J, Kagal A, Bharadwaj R. Factors associated with drug resistance in pulmonary tuberculosis. *Indian J Chest Dis Allied Sci* 2003; 45 : 105-9.

Kalpana S, Valladurai N, Ranganathan D, Manjula D; HIV positivity among multi-drug resistant tuberculosis patients. *Int Conf AIDS*. 2004 Jul 11-16; 15: abstract no. B11788.

Kameda K, Kawabata S., The chemotherapy of pulmonary tuberculosis complicated by diabetes mellitus. *Kekkaku,* 1986, 61(8):413–23.

Kamolratanakul P, Sawert H, Kongsin S, et al,Economic impact of tuberculosis at the household level. *Int J of Tuberc and Lung Disease*, 1999, 3(7):596–602.

Karachunskii MA, Komliakova EG, Posppelov LE.,Specific features of pulmonary tuberculosis course in patients with

insulin-dependent diabetes mellitus in relation to a varying HLA phenotype. *Problemy tuberkuleza*, 1997, 5:23–5.

Karak K, Bhattacharyya S, Majumdar S, De P.K. Pulmonary infections caused by mycobacteria other than M.tuberculosis in and around Calcutta. *Indian J Pathol Microbiol* 1996;39:131-4.

Katoch V.M. Infections due to Non Tuberculous Mycobacteria (NTM). *Indian J Med Res 2004;* 120:290-304.

Kemp J., Boxshall M., Nhlema B., Salaniponi F., Squire B.) Application of a Geographical Information System (GIS) to examine the relationship between socioeconomic status and access to care for TB in urban Lilongwe. *Int J Tuberc Lung Dis,* 2001, 5:11 Supplement 1:S167.

Kim SH, Hong YP, Lew WJ, et al. Incidence of pulmonary tuberculosis among diabetics. *Tuberc Lung Dis* 1995; 76: 529–523.

Kolappan C, Gopi P G. Tobacco smoking and pulmonary tuberculosis. *Thorax* 2002; 57: 964–966.

Liberato IR, Albuquerque F, Campelo AR, Melo H, Characteristics of pulmonary tuberculosis in HIV seropositive and seronegative patients in a northeastern region of Brazil as well as reactivation of latent tuberculosis infection. *Rev Soc Bras Med Trop* 2004; 37: 46-50.

Mahmoudi A, Iseman MD. Pitfalls in the care of patients with tuberculosis. Common errors and their association with the acquisition of drug resistance. *JAMA* 1993; 270: 65-8.

Malhotra, S Pathak, L Vyas, VM Katoch, K Srivastava, DS Chauhan, D Singh, VD Sharma, R Das, HB Singh. Drug susceptibility profiles of mycobacterium tuberculosis isolates at Jaipur, *Indian Journal of Medical Microbiology* 2002 Volume 20 Issue 2 Page: 76-78.

Mathur ML, PK Khatri, CS Base Drug resistance in tuberculosis patients in Jodhpur district *Indian Journal of medical sciences* 2000, Volume 54, Issue 2, Page: 55-8.

Mona Bashar, Phil Alcabes, William N. Rom and Rany CondosIncreased Incidence of Multidrug-Resistant Tuberculosis in Diabetic Patients on the Bellevue Chest Service, 1987 to 1997, *Chest* 2001;120;1514-1519.

Murray J, Sonnenberg P, Shearer S, Godfrey-Faussett P. Drug-resistant pulmonary tuberculosis in a cohort of southern African goldminers with high prevalence of HIV infection. *S Afr Med J* 2000; 90:381-6.

Narain JP, Raviglione MC, Kochi A. HIV associated tuberculosis in developing countries: epidemiology and strategies for prevention. *Int J Tuber Lung Dis* 1992; 73: 311-2.1.

Natal S, Toledo A, Penha MLF, Valente J. Modelo de predição para resistência aos tuberculostáticos [abstract]. *J Pneumol* 2000;26:S23.

Ngamvithayapong-Yanai J, Pungrassami P, Yanai H. Compliance with tuberculosis treatment: a gender perspective. In: Gender and Tuberculosis—An International Research Workshop. Göteberg, Sweden: *The Nordic School of Public Health*, 1998.

O'Boyle SJ, Power JJ, Ibrahim MY, Watson JP. Factors affecting patient compliance with anti-tuberculosis chemotherapy using the directly observed treatment, short-course strategy (DOTS). *Int J Tuberc Lung Dis.* 2002; 6(4):307-12.

Ormerod LP. Multidrug-resistant tuberculosis (MDR-TB): Epidemiology, prevention and treatment. *Br Med Bull* 2005;

73–74:17–24.

Pande J.N., U.B. Singh, Sanjeev Sinha ,R.C. Agarwal, SPN Singh, Evaluation of risk factors and prevalence of Drug resistant tuberculosis in North India, meeting.chestjournal.org/cgi/content/abstract/128/4/404S, 2005.

Pandit.N, S.K. Choudhary A Study of Treatment Compliance in Directly Observed Therapy for Tuberculosis *Indian Journal of Community Medicine* Vol. 31, No. 4, October-December, 2006-241.

Paramasivan C N, Govindan D, Prabhakar R, Somsundaran P, R, Subbammal S, Tripathy S . Species level identification of Non Tuberculous Mycobacteria from South Indian BCG trial area during 1981. *Tubercle* 1985; 66:9-15.

Paramasivan CN, Herbert D, Prabhakar R. Non-tuberculous mycobacteria: an overview. Lung India 1986; 4: 7-12.

Paramasivan CN, Venkataraman P, Chandrasekaran V, Bhat S, Narayanan PR. Surveillance of drug resistance in tuberculosis in two districts of South India. *Int J Tuberc Lung Dis*. 2002 Jun; 6(6):479-84.

Paramasivan, CN, An overview of drug-resistant tuberculosis in India. *Ind J tuberc* 1998; 45: 73 81.

Paramasivan CN, K. Bhaskarair, P. Venkataraman, V. Chandrasekaran and P.R. Narayanan Surveillance Of Drug Resistance In Tuberculosis In The State Of Tamil Nadu *Ind. J. Tub*, 2000, 47,27.

Park MH, Song EY, Park HJ, Kwon SY, Han SK, Shim YS. HLA-DRB1 and DQB1 gene polymorphism is associated with multidrug-resistant tuberculosis in Korean patients. *Hum Immunol* 2002; 63: S33.

Patel JC. Complications in 8793 cases of diabetes mellitus 14 years study in Bombay Hospital, Bombay, India. *Ind J Med Sci.* 1989, 43, 177.

Pathak SK, Deshmukh PA, Menon CRN. A comparison of different culture techniques. Indian J Tub 1973; 20: 85.

Pathania V, Almeida J, Kochi A. TB Patients and Private For-Profit Health Care Providers in India. Geneva, World Health Organization, *The Global TB Programme*, 1997. (Unpublished document WHO/TB/ 97.223)

Pauline Joseph, V. Chandrasekaran, A. Thomas, P.G. Gopi, R. Rajeswari, R. Balasubramanian,R. Subramani, N. Selvakumar and T. Santha.Influence of drug susceptibility on treatment outcome and susceptibility profile of 'failures' to CATEGORY II REGIMEN. *Indian J Tuberc* 2006; 53:141-148.

Pronab Chatterjee , Bratati Banerjee , Debashish Dutt, Rama Ranjan Pati,Ashok Kumar Mullick, A comparative evaluation of factors and reasons for defaulting in tuberculosis treatment in the states of West Bengal, Jharkhand and Arunachal Pradesh *Ind J Tub*, 2003,50, 17.

Pyle M.M. (1947) Relative numbers of resistant tubercle bacilli in sputum of patients before and during treatment with streptomycin. *Proc Mayo Clinic.* 22: 465-473.

Rajesh Mondal and Amita Jain, King George's Medical University, Lucknow, India, Extensively Drug-Resistant Mycobacterium tuberculosis, India Emerging Infectious Diseases, www.cdc.gov/eid Vol. 13, No. 9, September 2007.

RNTCP Status Report, Central TB Division, TB India 2008. Directorate General of Health Services, Ministry of Health and Family Welfare, Govt. of India, March 2008. http://www.tbcindia.org.

RNTCP Training Module for Medical Practitioners,Central TB Division 2006.

Ruddy M, Balabanova Y, Graham C, et al. Rates of drug resistance and risk factor analysis in civilian and prison patients with tuberculosis in Samara Region, Russia. *Thorax* 2005; 60: 130–135.

Ruder K. Fighting the epidemic: A United Nations resolution on diabetes. *Diabetes Forecast* 2007; 60:50–1.

Rupak Singla, Nazeer Khan, Rany Condos and Philip Alcabes Does Diabetes Predispose to the Development of Multidrug-Resistant Tuberculosis? , *Chest* 2003; 123; 308-309.

Santha T, R. Garg, T. R. Frieden, V. Chandrasekaran, R. Subramani, P. G. Gopi, N. Selvakumar, S. Ganapathy, N. Charles, J. Rajamma, P. R. Narayanan, Risk factors associated with default, failure and death among tuberculosis patients treated in a DOTS programme in Tiruvallur District, South India, *Int J Tuberc Lung Dis 2000;* 6(9):780–788.

Santha T, Renu G, Frieden TR, et al. Are community surveys to detect tuberculosis in high prevalence areas useful? Results of a comparative study from Tiruvallur District, South India. *Int J Tuberc Lung Dis* 2002; 7:258-265.

Santha T, P.G. Gopi, R. Rajeswari, N. Selvakumar, R. Subramani, V. Chandrasekaran, B. Rani, A. Thomas and P.R. Narayanan Is It Worth Treating Category I Failure Patients With Category II Regimen? *Indian J Tuberc* 2005; 52:203-206.

Sarman Singh, Sankar, Manimuthu Mani, Gopinath , Krishnamoorthy, High rate of extensively drug-resistant tuberculosis in Indian AIDS patients. *AIDS.* 21(17):2345-2347, November 2007.

Shah, A.R.Agarwal, S. K. & Shah, K. V. Study of drug resistance in previously treated tuberculosis patients in Gujrat, India. *Int J Tuberc Lung Dis 2002;* 6, 1098–1101.

Sharma SK, Turaga KK, Balamurugan A, Saha PK, Pandey RM, Jain NK, et al. Clinical and genetic risk factors for the development of multidrug-resistant tuberculosis in non-HIV infected at a tertiary care center in India: a case-control study. *Infect Genet Evol* 2003; 3: 183-8.

Sharma S.K &. Mohan.A.,,Multidrug-resistant tuberculosis, *Indian J Med Res* 120, October 2004, pp 354-376.

Sharma SK, Mohan A.Multidrug-resistant tuberculosis: a menace that threatens to destabilize tuberculosis control. *Chest.* 2006 Jul; 130(1):261-72.

Shimao T. Drug-resistance in tuberculosis control. *Tubercle* 1987; 68 (suppl):5-15.

Singh V., Jaiswal A., Porter J.D.H., Ogden J.A., Sarin R., Sharma P.P., Arora V.K., Jain R.C. (2002) TB control, poverty, and vulnerability in Delhi, India. *Tropical Medicine and International Health* 7(8):693-700.

Sivasankari1 P., Annie B. Khyriem, K. Venkatesh and Subhash Chandra Parija ,Atypical Mycobacterial Infection Among HIV Seronegative Patients in Pondicherry .Indian J Chest Dis Allied Sci 2006; 48: 107-109.

Small PM, Shafer RW, Hopewell PC, Singh SP, Murphy MJ, Desmond E, et al. Exogenous reinfection with multidrug-resistant Mycobacterium tuberculosis in patients with advanced HIV infection. *N Engl J Med* 1993; 328.

Sonnenberg P, Murray J, Glynn JR, Shearer S, Kambashi B, Godfrey-Faussett P. HIV-1 and recurrence, relapse, and reinfection of tuberculosis after cure: A cohort study in South African mineworkers. *Lancet* 2001; 358:1687-93.

Sophia Vijay, Balasangameshwara, Jagannatha,, Saroja, V.N., Shivashankar, B., Jagota, P.: Retreatment outcome of smear positive tuberculosis cases under DOTS un Bangalore City, *Ind J Tub*, 2002; 49:195-204.

Sophia Vijay, VH. Balasangameswara, Jagannatha, VN Saroja and P Kumar, Defaults Among Tuberculosis Patients Treated Under Dots In Bangalore City : A Search For Solution *Ind. J Tub.*, 2003, 50:185.

Spellman CW, Matty KJ, Weis SE. A survey of drug resistant Mycobacterium tuberculosis and its relationship to HIV

infection. *AIDS* 1998; 12: 191-5.

Subhash H. S, Ashwin I., Mukundan U, Danda D, John G, Cherian A.M., Thomas K. ; Tropical Doctor ISSN 0049 55 CODEN TPDCAV 2003, Vol. 33, No.3, Pp. 154-156.

Subodh K Katiyar, Shailesh Bihari, S Arun, Tara Rawat, An Analysis Of Failure Of Category II DOTS Therapy Indian Journal Of Community Medicine 2008, Vol. 33, Issue 2.

Sudha Ganapathy, Chandrasekaran V, Britto JJ, Jemima SF, Cahrles N, Santha T, Sudarsanam NM, Prabhakar R. A study of patients 'lost' from short course chemotherapy under the district tuberculosis programme in south India. *Lung India* 1994; **3**: 129-134.

Sumartoyo E. When tuberculosis treatment fails. A social behavioural account of patient adherence. *American review of respiratory diseases*, 1993, 147:1311-20.

Swaminathan S, C. N. Paramasivan, C. Ponnuraja, S. Iliayas, S. Rajasekaran, P. R. Narayanan Anti-tuberculosis drug resistance in patients with HIV and Tuberculosis in South India, *Int J Tuberc Lung Dis* 9(8):896–900, 2005.

Tekle B, Mariam DH, Ali A. Defaulting from DOTS and its determinants in three districts of Arsi Zone in Ethiopia. *Int J Tuberc Lung Dis.* 2002 6(7):573-9.

Temple C.W., Hughs E.J., Mardis R.E., et al. (1951) Combined intermittent regimens employing streptomycin and para-aminosaicylic acid in the treatment of pulmonary tuberculosis. *Am Rev Tuber.* 63: 295-311, in the United States and the British Thoracic Society (MRC, 1951).

Thomas, A literature review of the problems of delayed presentation for treatment and non-completion of treatment for tuberculosis in less developed countries and ways of addressing these problems using particular implementations of the DOTS strategy. Review *J Manag Med.* 2002; 16(4- 5):371-400.

Thomas, R. Rajeswari, S. Anandakrishnan, M. Perumal, C. Niruparani, G. Sudha, Jaggarajamma, T. R. Frieden, P. R. Narayanan. Gender disparities in tuberculosis: report from a rural DOTS programme in south India *Int J Tuberc Lung Dis 2004;* 8(3):323–332.

Thomas, Gopi PG, Santha T, et al. Predictors of relapse among pulmonary tuberculosis patients treated in a DOTS programme in South India. *Int J Tuberc Lung Dis* 2005; 9:556-561.

Toman.K , What is the probability of obtaining a negative culture from a sputum specimen found positive by smear microscopy, pg 44-45, Toman's Tuberculosis, II edition, WHO, Geneva.

Topley and Wilsons Microbiology and Microbial Infections, Bacteriology-2, Tenth edition p.1186 & 1202.

Trivedi SS, Desai SC. Primary Antituberculosis drug resistance and acquired Rifampicin resistance in Gujarat -India. *Tubercle* 1988; 69:37-42.

Tsukaguchi K et al., Longitudinal assessment of IFN-gamma production in patients with pulmonary tuberculosis complicated with diabetes mellitus, *Kekkaku*, 2002, 77(5):409–13.

Uplekar MW, Rangan S. Tackling TB: Search for Solutions. The Foundation for Research in Community Health: *Bombay*; 1996.

Uplekar, S. Juvekar, S. Morankar, S. Rangan, P. Nunn. Tuberculosis patients and practitioners in private clinics in India, *Int J of Tuberc and Lung Dis*,Volume2, Number 4, April 1998, pp. 324-329(6).

Uplekar MW, Pathania V, Raviglione M. Private practitioners and public health: weak links in tuberculosis control. *Lancet.* 2001; 358:912-6.

Vargas, Bernabé A, Gilman R.H, Kawai V, Soto G, Moore D.A, Caviedes L, Factors associated with multidrug-resistant tuberculosis in HIV -infected patients, Peru Int Conf AIDS. 2006 Aug 13-18; 16 Abstract No. MoAb0103.

Vasanthakumari R; Jagannath K, Multidrug resistant tuberculosis -A Tamilnadu study. *Lung India.* 1997 Oct-Dec; 15(4): 178-80.

Verbon A, Leemans JC, Weijer S, Florquin S, Van Der Poll T. Mice lacking the multidrug resistance protein 1 have a transiently impaired immune response during tuberculosis. *Clin Exp Immunol* 2002; 130 : 32-6.

Wallace R J Jr, O'Brein R, Glassroth J, Raleigh J, Dutta A. Diagnosis and treatment of disease caused by Non Tuberculous Mycobacteria. *Am Rev Respir Dis* 1990; 142:940-53.

Wang CH et al. Hypodense alveolar macrrophages in patients with diabetes melllitus and active pulmonary tuberculosis. *Tuberculosis and lung diseases*, 1999, 79(4):235–42.

Weyer K, Kleeberg HH. Primary and acquired drug resistance in adult black patients with tuberculosis in South Africa: results of a continuous national drug resistance surveillance programme involvement. *Tuberc Lung Dis* 1992; 73: 106 -12.

WHO report 2007. Global tuberculosis control: surveillance, planning, financing. Available at: http://www who int/tb/publications/global_ report/2007/pdf/full.pdf.

WHO Tuberculosis fact sheet 2008 available from URL:http://www.who.int/gtb/publications/factsheet/index.htm.

Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004; 27:1047–53.

Youmans G.P., Williston E.H., Feldman W. and Hinshaw C.H. Increase in resistance of tubercle bacilli to streptomycin. A preliminary report. Proc Mayo clinic 1946; 21: 126.

Annexure –I

(Media preparation)

ANNEXURE I

PREPARATION OF 4% NaOH :

Dissolve 4 gm of sodium hydroxide in 100 ml of distilled water. Sterilize by autoclaving. Kept at 37 °C until use.

PREPARATION OF 1%CPC-NaCl:

One gm of cetylpyridinium chloride and two gms of sodium chloride are dissolved in 100ml of sterile distilled water and distributed in 5 ml aliquots in sterile MacCartney bottles. The stock solution should be stored in dark coloured bottles at room temperature.

PREPARATION OF LOWENSTEIN-JENSEN MEDIUM:

Preparation of Mineral salt solution with malachite green:

Dissolve 37.24 gms of Himedia Lowenstein- Jensen media base and 12 ml Glycerol in 600 ml distilled water. Sterilize by autoclaving.

Homogenised whole eggs:

Fresh country hen's eggs, those are not more than seven days old, are cleaned by scrubbing thoroughly with a hand brush in water and soap. Let the eggs soak for 30 minutes in soap solution. Rinse eggs thoroughly in running water and soak them in 70% ethanol for 15 minutes. Crack the eggs into a sterile flask and beat them in a sterile blender.

Preparation of complete medium:

The following ingredients are aseptically pooled in a large, sterile flask and mixed well:

Mineral salt solution with malachite green- 600ml

Homogenized eggs (25-30 eggs, depending on size) 1000ml

The complete egg medium is distributed in 6-8ml volumes in sterile MacCartney bottles and the caps tightly closed and inspissated without delay to prevent sedimentation of heavier ingredients.

Sterility check:

After inspissation, the whole batch of the media bottles should be incubated 37 deg C for 24 hours as a check of sterility. After 24 hours 5% of the slopes should be picked up randomly and continued incubation. In both the cases the contamination rate should not be more than 10 %.

Storage:

The LJ medium should be dated and stored with the batch number in the refrigerator and can be kept for up to 4 weeks if the caps are tightly closed to prevent drying out of the medium.

Medium Containing P-Nitrobenzoic Acid

Weigh out 0.5 gm PNB and dissolve in the minimum amount of dimethylformamide (~15ml). Add to 1 litre of L-J fluid, distribute and inspissate once for 50 minutes at 85 °C. Store at 4 °C.

Niacin test:

Reagents

1) O-toluidine - 1.5% (O-toluidine - 1.5 g and Ethanol -100ml)

Mix in an amber coloured bottle and store it in the dark, in a refrigerator, prepare fresh weekly.

2) Cyanogen bromide solution, approx. 10%.

Store at 4 ° C in the refrigerator.

Preparation of drug containing LJ media:

1. Isoniazid

Stock solution preparation:

Weigh out 200 mg of Isoniazid in 20ml of sterile distilled water (10,000 µg/ml). Dissolve and sterilize by membrane filtration. Keep filtered solution frozen up to one month.

Working solution preparation:

Sl.No.	Stock Solution	Distilled Water(Ml)	Concentration(µg/ml)
1.	1 ml of 10,000 µg/ml	9	1000
2.	2 ml of 1000 µg/ml	18	100
3.	1 ml of 100 µg/ml	19	5
4.	2 ml of 5 µg/ml	8	1

Media preparation:

Sl.No.	Stock Solution	LJ Fluid(ml)	Final Concentration(µg/ml)
1.	2.5 ml of 1 µg/ml	100	0.025
2.	5 ml of 1 µg/ml	100	0.05
3.	2 ml of 5 µg/ml	100	0.1
4.	1.2 ml of 100µg/ml	600	0.2
5.	6 ml of 100 µg/ml	600	1.0
6.	3 ml of 1000 µg/ml	600	5.0

2. Rifampicin

Stock solution preparation:

Weigh out 200 mg of Rifampicin in 20ml of dimethyl formamide (10,000 µg/ml).

Working solution preparation:

Sl.No.	Stock Solution	Distilled Water(ml)	Concentration(µg/ml)
1.	4 ml of 10,000 µg/ml	16	2000
2.	2 ml of 2000 µg/ml	18	200

Media preparation:

Sl.No.	Stock Solution	LJ Fluid(ml)	Final Concentration(µg/ml)
1.	7.68 ml of 10,000 µg/ml	600	128
2.	3.84 ml of 10,000 µg/ml	600	64
3.	9.6 ml of 2000 µg/ml	600	32
4.	8 ml of 200µg/ml	100	16
5.	4 ml of 200 µg/ml	100	8
6.	2 ml of 200 µg/ml	100	4

3. Ethambutol

Stock solution preparation:

Weigh out 270 mg of Ethambutol in 20ml of distilled water (10,000 µg/ml). Dissolve and sterlize by membrane filtration.

Working solution preparation:

Sl.No.	Stock Solution	Distilled Water(ml)	Concentration(µg/ml)
1.	2 ml of 10,000 µg/ml	18	1000
2.	10 ml of 1000 µg/ml	10	500
3.	1 ml of 500 µg/ml	9	50

MEDIA PREPARATION:

Sl.No.	Stock Solution	LJ Fluid(ml)	Final Concentration(µg/ml)
1.	1ml of 50 µg/ml	100	0.5

2.	2 ml of 50 µg/ml	100	1.0
3.	2.4 ml of 500 µg/ml	600	2.0
4.	4.8 ml of 500µg/ml	600	4.0
5.	4.8 ml of 1000 µg/ml	600	8.0

4. Streptomycin

Stock solution preparation:

Weigh out 250 mg of Streptomycin in 20ml of distilled water (10,000 µg/ml). Dissolve and sterilize by membrane filtration.

Working solution preparation:

Sl.No.	Stock Solution	Distilled Water(ml)	Concentration(µg/ml)
1.	4 ml of 10,000 µg/ml	16	2000
2.	1 ml of 2000 µg/ml	19	100

Media preparation:

Sl.No.	Stock Solution	LJ Fluid(ml)	Final Concentration(µg/ml)
1.	2ml of 100 µg/ml	100	2
2.	4 ml of 100 µg/ml	100	4
3.	2.4 ml of 2000 µg/ml	600	8
4.	4.8 ml of 2000µg/ml	600	16
5.	9.6 ml of 2000 µg/ml	600	32
6.	3.84 ml of 10,000 µg/ml	600	64

5. Kanamycin:

Stock solution preparation:

Weigh out 128 mg of Kanamycin in 10ml of distilled water (10,000 µg/ml). Dissolve and sterlize by membrane filtration.

Working solution preparation:

Sl.No.	Stock Solution	Distilled Water(ml)	Concentration(µg/ml)
1.	4 ml of 10,000 µg/ml	6	4000
2.	2 ml of 10,000µg/ml	18	1000
3.	1 ml of 1000 µg/ml	19	50

Media preparation:

Sl.No.	Stock Solution	LJ Fluid(ml)	Final Concentration(µg/ml)

1.	2ml of 50 µg/ml	50	2
2.	4 ml of 50 µg/ml	50	4
3.	2.4 ml of 1000 µg/ml	300	8
4.	4.8 ml of 1000µg/ml	300	16
5.	2.4 ml of 4000 µg/ml	300	32
6.	4.8 ml of 4000 µg/ml	300	64

6. Ethionamide:

Stock Solution Preparation:

Weigh out 200 mg of Ethionamide in 20ml of tri-ethylene glycol. Mix well and incubate overnight for self sterilization (10,000 µg/ml). Do not sterlize by membrane filtration.

Working solution preparation:

Sl.No.	Stock Solution	Distilled Water(ml)	Concentration(µg/ml)
1.	6 ml of 10,000 µg/ml	14	3000
2.	8.55 ml of 10,000µg/ml	11.45	4275

Media preparation:

Sl.No.	Stock Solution	LJ Fluid(ml)	Final Concentration(µg/ml)
1.	2ml of 3000 µg/ml	300	20
2.	2 ml of 4275 µg/ml	300	28.5
3.	4 ml of 3000 µg/ml	300	40
4.	4 ml of 4275µg/ml	300	57
5.	8 ml of 3000 µg/ml	300	80
6.	8 ml of 4275 µg/ml	300	114

7. Ofloxacin:

Stock Solution Preparation:

Weigh out 100 mg of Ofloxacin in 10ml of sterile 0.1 N NaOH (10,000 µg/ml).

Working solution preparation:

Sl.No.	Stock Solution	Distilled Water(ml)	Concentration(µg/ml)
1.	2 ml of 10,000 µg/ml	18	1000
2.	1 ml of 1000µg/ml	19	50

Media preparation:

Sl.No.	Stock Solution	LJ Fluid(ml)	Final Concentration(µg/ml)
1.	1ml of 50 µg/ml	100	0.5
2.	2 ml of 50 µg/ml	100	1.0
3.	1.2 ml of 1000 µg/ml	600	2.0
4.	2.4 ml of 1000 µg/ml	600	4.0
5.	4.8 ml of 1000 µg/ml	600	8.0

Annexure –II

(Proforma of the Data sheet)

ANNEXURE II

DATA SHEET FOR COLLECTION OF SOCIO DEMOGRAPHIC, CLINICAL AND LABORATORY DATA

FOR P.G. DISSERTATION WORK ON

"EVALUATION OF THE DRUG SUSCEPTIBILITY PROFILE AMONG CATEGORY II PULMONARY TUBERCULOSIS PATIENTS (RELAPSE, FAILURE, DEFAULTER) AT TIRUNELVELI"

Name: Age/Sex:

Address: Rural/ Urban

Occupation: Income:

Education:

Smoking habit:

H/O PRESENT ILLNESS:

COUGH: Duration

Dry/ productive

Purulent/ watery/mucoid

Haemoptysis

FEVER: Duration

Continuous/ Intermittent.

Evening rise of temperature.

LOSS OF APPETITE:

LOSS OF WEIGHT:

WHEEZING:

CHEST PAIN: Site

Pricking/ compressing

Radiating

Associated with cough

BREATHLESSNESS: mild / moderate / severe exertion

TREATMENT HISTORY:

[FOR PATIENTS STARTED ON TMT/ CATEGORY II FAILURES]

CATEGORY I/ II/ III

CATEGORY II- RELAPSE / DEFAULTER / FAILURE.

DOTS/ NON DOTS:

Treatment started on:

At which hospital:

Sputum results:

Month	Date	DMC	Lab No.	Smear result	Weight
Pretreatment					
End IP/Extended IP					
2 months CP					
End treatment					

Treatment outcome:

Cured/ Completed/ Failure/ Defaulted/Transferred out/ Died

Patient follow up:

PAST HISTORY:

H/o previous Anti TB treatment: YES / NO

If YES:

CATEGORY I/ II / III/ NOT KNOWN

How long back:

At which hospital: PRIVATE / GOVT

Sputum results: positive/ negative/not known.

Duration of treatment:

Regular/ irregular:

Defaulted:

Reason for default:

Treatment outcome: Cured/ Completed/ Failure/ Transferred out/ Died

Further management:

Treatment history if available from previous records:

H/o Diabetes/ Bronchial asthma/Others

Treatment particulars:

FAMILY HISTORY:

Family members:

Any other family member taking tmt for Tuberculosis:

Are they suffering from any signs suggestive of TB?

INVESTIGATIONS:

BLOOD SUGAR- RANDOM

-FASTING

-POSTPRANDIAL

HIV STATUS: [AS PER ICTC REPORTS]

SPUTUM PROCESSED ON:

CULTURE READING: ---------- WEEKS * / + / ++ / +++

* CONTAMINATION

+++ CONFLUENT GROWTH

++ INNUMERABLE DISCRETE COLONIES

\+ 20-100 COLONIES

NIACIN TEST:

GROWTH IN PNB:

DRUG SENSITIVITY RESULT:

S / R – STREPTOMYCIN

S / R – ISONIAZID

S / R – RIFAMPICIN

S / R – ETHAMBUTOL

SECOND LINE DRUG TESTING:

S / R – KANAMYCIN

S / R –ETHIONAMIDE

S / R – OFLOXACIN

INFERENCE: