DRUG RESISTANCE PATTERNS OF *MYCOBACTERIUM TUBERCULOSIS* COMPLEX AND ASSOCIATED FACTORS AMONG RETREATMENT CASES AT JIMMA UNIVERSITY SPECIALIZED HOSPITAL, SOUTH WEST ETHIOPIA

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A RESEARCH THESIS SUBMITTED TO DEPARTMENT OF MEDICAL LABORATORY SCINCES AND PATHOLOGY, COLLEGE OF PUBLIC HEALTH AND MEDICAL SCIENCES JIMMA UNIVERSITY FOR THE PARTIAL FULFILLMENT OF REQUIREMENT FOR MASTERS DEGREE IN CLINICAL LABORATORY SCIENCE SPECIALITY IN CLINICAL MICROBIOLOGY.

SEPTEMBER 2013
JIMMA ETHIOPIA

JIMMA UNIVERSITY
COLLEGE OF PUBLIC HEALTH AND MEDICAL SCIENCES
DEPARTMENT OF MEDICAL LABORATORY SCIENCES AND PATHOLOGY

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8.1.

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1. **Gemeda Abebe (MSc, PhD)**
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ABSTRACT

Background: The global burden of tuberculosis(TB) has been accompanied with the emergence of multidrug-resistant tuberculosis(MDR-TB).The rate of MDR-TB is five times higher among previously treated tuberculosis cases than new cases. There is a need to determine the level of drug resistant TB among these patients. **Objective:** The objective of this study was to determine drug resistance patterns of *Mycobacterium tuberculosis* complex isolates among previously treated TB cases at Jimma University specialized Hospital, south west Ethiopia.

Methods: A prospective cross-sectional study was conducted from March 2012 to April 2013. A total of 79 smear positive previously treated TB cases were enrolled in to the study. Structured questionnaire and patients medical records were used to collect participant's socio-demographic and clinical data. Sputum specimens were collected and cultured in to the modified middlebrook 7H9 broth media. Identification of *Mycobacterium tuberculosis* complex was done by paranitrobenzoic acid (500 μg/ml) inhibition test. Drug susceptibility testing was done to four first line drugs (streptomycin 1μg/ml , isoniazid 0.1μg/ml , rifampicin 1μg/ml and ethambutol 5μg/ml) in modified middlebrook 7H9 broth media by BACTEC /MGIT 960 system using indirect proportion method. Data were analyzed using SSPS version20.

Results: 70 *Mycobacterium tuberculosis* complex isolates were tested for drug susceptibility patterns. Out of these 41/70 (58.6%) isolates were resistant to one or more drugs. Any drug resistance to isoniazid 36/70 (51.4%) was found to be the most common. Factors associated with any drug resistance were, residence place (AOR: 3.44, 95%: CI 1.11, 10.60, p=0.032), duration of illness (AOR: 3.4, 95%: CI 1.10, 10.62, p=0.035) and number of treatment before this episode (AOR: 2.99, 95%: CI 1.01, 8.86, p=0.048). The prevalence of MDR-TB was 22/70(31.4%). Patients with the history of treatment failures were 3.4 times more likely to have MDR-TB than relapse cases (AOR: 3.43, 95% CI: 1.14, 10.28, p=0.028). All of the MDR-TB isolates were resistant to either streptomycin or ethambutol. A total of 12/70(17.1%) MDR-TB isolates were resistant to all four first-line drugs. About 97.5% of rifampicin resistant isolates were MDR-TB.

Conclusion and recommendation: In this study**,** the prevalence of any drug resistance and MDR-TB were high. MDR-TB showed statistically significant association treatment failures. Patients' compliance to full course therapy should be supervised .Treatment failures should timely be identified and referred for culture and drug susceptibility testing.

Key words: Mycobacterium tuberculosis complex, drug resistant M.tuberculosis ,MDR-TB

TABLE OF CONTENTS

LIST OF TABLES

ACKNOWLEDGEMENT

I express my sincere and deepest gratitude to my advisors Dr. Gemeda Abebe and Mr.Ketema Abdissa for introducing me into the world of mycobacteriology and for their regular supervision and advice. This research work was done at Jimma University core laboratories of mycobacteriology laboratory as part the Interuniversity Cooperation Jimma University (IUC-JU) programme. Therefore, I would like to thank the managements of IUC-JU particularly the IUC-JU TB/HIV sub-project research team leader. I would like to thanks Jimma University for sponsoring my education, Department of Medical laboratory science and pathology for giving me chance to do my research thesis in on this title and Jimma University College of Public Health and Medical science office of postgraduate and research for financial support.

I would like to express my deep gratitude to the department of medical laboratory science and pathology that enrolled me in my MSc study and helped me in different ways to finish my thesis. I wish to express my sincere gratitude to Mr Ahmed Zeynidin. My gratitude also goes to my colleagues and friends at Jimma University Mycobacteriology laboratory; Mr. Mulualem Tedesse, Mr Belayneh Dimah , Mr Tufa fayisa and Mr .Feyisa Ifa for their help in the activities of data collection and laboratory works.

I am grateful to Mr Desta Hiko who helped me in analyzing the data. I would like to express my gratitude to all staffs of the department and my friends for their continuous help and support. it is my great pleasure to express my sincere gratitude to my family for their patience and continued support. Finally I would like to address my great thank and respect to study participants for their voluntary take part in this study.

LIST OF ABBREVIATIONS AND ACRONYMS

AFB = Acid fast bacilli

BCG=Bacillus Calmette–Guérin

AIDS= Acquired immunodeficiency syndrome

DST= Drug susceptibility testing

DOTS: Directly Observed Treatment, Short-course

DR-TB=Drug resistant tuberculosis

EMB=Ethambutol

EPTB=extrapulmonary tuberculosis

GC= Growth control

HIV= Human immunodeficiency virus

INH=Isoniazid

IUATLD=International union against tuberculosis and lung disease.

LJ media=Lowenstein-Jensen media

MDR-TB= Multidrug resistant tuberculosis

MGIT=Mycobacteria growth indicator tube

M.tuberculosis*=Mycobacterium tuberculosis***

MTBC=*Mycobacterium tuberculosis* complex

NTM =Non-tuberculosis mycobacteria

OADC= Oleic acid albumin dextrose catalase

PCR= Polymerase chain reaction

PNB=Paranitrobenzoic acid

PTB=Pulmonary tuberculosis

PZA=pyrazidamine

RIF= Rifampcin

TB=Tuberculosis

STP = Streptomycin

WHO= World health organization

XDR-TB= Extensively drug resistant tuberculosis

SUMMARY

Tuberculosis remains the leading infectious disease causing significant morbidity and mortality worldwide. World Health Organization (WHO) had estimated that one third of the world population is latently infected with *M. tuberculosis*. Among these 8 million people develop active TB and 2 million people die of TB each year. The emergence of DR-TB/MDR-TB poses a serious risk of compromising the effectiveness of TB elimination plan of 2050 (<1 case per 1 million population. Establishing the level of drug resistant *M.tuberculosis* complex among previously treated TB patients is an important aspect of TB control programme. Therefore, the finding of this study will helpful in determining the level of drug resistant *M.tuberculosis* complex among previously treated cases in study setting

CHAPTER ONE

INTRODUCTION

1.1. TUBERCULOSIS

1.1.1 Microbiology of Tuberculosis

Bacteria of the genus *Mycobacteria* are non-motile and non-sporulated rods. These are grouped in the suprageneric rank of actinomycetes that unusually have a high content (61-71 %) of guanine plus cytosine (G+C) in their genomic DNA. Structurally, *Mycobacteria* are slender, curved rods, resistant to acids, alkalis and dehydration[2, 3].

The most distinctive feature of genus *Mycobacterium* is its cell wall composition. Although the basic structure of the cell wall is typical of gram-positive bacteria, it has distinctive complex lipid rich cell wall. This complex cell wall is responsible for many natural characteristics of the genus; such as acid fastness, slow growing rate, resistance to detergents and resistance to common antibacterial antibiotics and cord formation[4].

The genus *Mycobacterium* is classified into members of the *Mycobacterium tuberculosis* complex (*M. tuberculosis, M. bovis, M. bovis* BCG, *M. africanum, M. microtii M. pinnipedii* and *M.caprae*) and *Mycobacteria* other than tuberculosis complex. This classification is commonly based on the rate of growth, pigment production in light and/or dark and different biochemical tests, such as catalase test, niacin production and paranitrobenzoic acid inhibition test[2, 3]. Most members of *Mycobacterium tuberculosis* complex and few groups of *Mycobacteria* other than tuberculosis complex have been found to cause tuberculosis (TB). However, *M.tuberculosis* is the most common causative agents of TB in human[5] .

1.1.2 Transmission and pathogenesis of *Mycobacterium tuberculosis*

M.tuberculosis transmits via respiratory system. Tubercle bacilli containing droplet nuclei of 1–5 microns are generated from patients with pulmonary or laryngeal TB disease when they are coughing, sneezing and even talking. These thinly particles are carried in air and suspended in the environment for several hours. Infection occurs when healthy person inhales tubercle bacilli containing droplet nuclei that reaching to the alveoli of the lungs. The probability of infection

and progression in to active disease is depends on different factors, like host susceptibility, infectiousness of index cases, environment and extent of exposure[6].

Inhaled tubercle bacilli in the droplet nuclei are engulfed by alveolar macrophages. The majority of these bacilli are destroyed by alveolar macrophages. However, bacilli are completely eradicated in only 10% of infected people. Under normal conditions, the bacilli are contained by immune response in 90% of infected people. In 5-10 % of cases, the bacilli escape microbicidal mechanisms of alveolar macrophage by inhibiting phagosome-lysosome fusion, antigen presentation, nitricoxide action and other reactive nitrogen intermediates action [5, 6].

Hence, in about 10% of infected people the bacilli multiply intracellularly. The multiplied bacilli are released when the infected macrophages die to infect other macrophages.This primary infection and active multiplication of the bacilli leads to clinical disease. In the remaining 90% of infected cases, the ensuing immune response arrests multiplication and dissemination of the bacilli, resulting in latent tuberculosis infection (*LTBI*). Latently infected people have high probability of developing active disease, if they are exposed to predisposing factors for TB reactivation [5]. In certain conditions particularly in immunocompromized individuals the bacilli can spread through lymphatic channels or bloodstream to other tissues and organs to cause extra-pulmonary tuberculosis(EPTB)[7].

1.1.3. Clinical Manifestation of tuberculosis

When patient progresses to active tuberculosis, early signs and symptoms are often nonspecific. Manifestations often include progressive fatigue, malaise, unintentional weight loss and a low-grade fever accompanied by chills and night sweats [8]. Wasting, a classical feature of TB is due to the lack of appetite and the altered metabolism associated with the inflammatory and immune responses. Wasting involves the loss of both fat and lean tissue. Decreasing in muscle mass leads to the fatigue [9]. Finger clubbing, a late sign of poor oxygenation may occur. However, it does not indicate the extent of disease. Cough eventually develops in most patients and initially may not be productive. Up on progression of the disease it becomes productive of purulent sputum. The sputum may also be streaked with blood which may due to destruction or dilated blood vessels or the formation of an aspergilloma in an old cavity [10].

1.1.4. Global Epidemiology of tuberculosis

Tuberculosis remains the leading infectious disease causing significant morbidity and mortality worldwide[1]. World Health Organization (WHO) had estimated that one third of the world population are latently infected with *M. tuberculosis*. Among these 8 million people develop active TB and 2 million people die of TB each year[11] .The TB mortality rate dropped 41% between 1990 and 2011. However, the global burden of TB remains enormous. According to WHO report of 2012, in 2011 there were an estimated 8.7 million new cases of TB (13% co-infected with HIV). About 1.4 million people died from TB, including almost one million deaths among HIV-negative individuals and 430 000 among people who were HIV-positive. TB is one of the top killers of women, with 300 000 deaths among HIV-negative women and 200 000 deaths among HIV-positive women[12, 13].

Global TB progression conceals with regional variations[1].The Africa and European regions are not on track to halve 1990 levels of mortality by 2015[13].This variation is accompanied with the socio-economic and hygienic conditions of human populations. Therefore, TB incidence rate is higher in Asia, Africa, and some parts of Latin America where poverty, infectious disease, poor health infrastructure and malnutrition are the most common problem of the human population.The persistence of high TB burden in the regions with the weak health infrastructure has exacerbated with the epidemicity of HIV/AIDS in the regions[14, 15].

Human immune deficiency virus (HIV) and TB form a lethal combination, each speeding the other's progress. HIV infection is a potent risk factor for TB. Not only does HIV increase the risk of reactivating latent *M. tuberculosis* infection, it also increases the risk of rapid TB progression soon after *M. tuberculosis* infection or re-infection. In persons infected with *M. tuberculosis* only, the lifetime risk of developing active TB disease ranges between 10 %and 20%. In persons co-infected with *M. tuberculosis* and HIV, however, the annual risk can exceed 10 %[2].

1.1.4.1 Tuberculosis in Ethiopia

Ethiopia is ranked seven in the list of 22 high TB burden countries. The WHO report showed that there were an estimated 220,000 (258 per 100,000 populations) incident cases of TB in Ethiopia. The prevalence of TB was estimated to be 200,000 (237 per 100,000 populations[13].

Federal ministry of health report showed that tuberculosis is the second cause of hospital death in Ethiopia. In 2010/11, a total 159,017 TB cases were notified in Ethiopia .Out of these 151,866 (95.5%) were new cases all forms of TB. The proportions of new smear-positive, smear negative pulmonary tuberculosis (PTB) and EPTB among all new cases are 32.7%, 34.8%, and 32.5% respectively. Retreatment cases accounted for 2.9% of all TB cases [16].

The 2009/2010 annual TB and leprosy prevention and control report of Jimma zone showed that, TB diagnostic and treatment services have been provided in 59.3% of health facilities. Case notification rates were 94 per 100000 populations for all forms of TB and 32 per 100000 populations for smear positive PTB. Case detection rates were 32.5% and 26.3% for all forms of TB and smear positive PTB respectively. The proportion smear positive PTB, smear negative PTB and EPTB were 40%, 28% and 32% respectively [17].

1.1.5. Drug Resistance in *Mycobacterium tuberculosis*

Drug resistance in *M.tuberculosis* is defined as a decrease in sensitivity of sufficient degree to be reasonably certain that the strain concerned is different from a sample of wild strains of human type that have never come in to contact with the drugs[18]. Over the past decades, the tuberculosis bacilli became resistant to various anti-tuberculosis drugs, making infection control increasingly difficult.Global surveillance has shown that drug resistant tuberculosis is widespread and is now a threat to tuberculosis control programs in many countries[19].

Drug resistance in *M.tuberculosis* is a man-made amplification of a natural phenomenon. Unlike the situation in many other bacteria, there is no horizontal transfer of gene for drug resistance. Drug resistance in *M.tuberculosis* starts with spontaneous drug resistance-conferring mutations in *Mycobacterial* genomic DNA. However, spontaneous mutations occur at a low frequency of 10^{-6} to 10^{-8} per cell replications. This can occur in the absence of anti-tuberculosis drugs. Therefore, drug resistant *M.tuberculosis* can find in naive patients who have never been treated with anti- tuberculosis drugs. Under effective therapy, naturally occurred few drug resistant *M.tuberculosis* strains are diluted by the majority of drug-susceptible bacterial populations [20, 21].

However, an interrupted or an inadequate therapy (due to prescription error, poor quality drugs and poor adherence or combination of these) provides the selective pressure for resistant strains to become dominant over the susceptible strains. This is most common in patients with a large load of bacilli [21, 22]**.** This amplification of resistance (acquired resistance*)* and subsequent transmission can worse the problems of drug resistance. Inappropriate therapies also enhance the acquisition of further drug resistance from initial mono drug resistance. The accumulation of strains with resistance against many anti-tuberculosis drugs may result in multi-drug resistant tuberculosis(MDR-TB) or extensively drug resistant tuberculosis(XDR-TB)[23].

Multi-drug resistant tuberculosis (MDR-TB) is defined as resistance of *Mycobacterium tuberculosis* to at least isoniazid (INH) and rifampicin (RIF) the most potent first-line anti-tuberculosis drugs. The emergence of MDR-TB has become additional challenges to TB control programme. Patients with MDR -TB poorly respond to the first-line anti-tuberculosis drugs. Their treatment with second-line drugs for a long duration often requires hospitalization for management of toxic reactions and other complications from the drugs. This puts constraints on meager health care resources in developing countries [24, 25]. Furthermore, the emergence of XDR-TB, defined as MDR-TB plus resistance to any of the fluoroquinolones and at least one of the three injectable second-line drugs (kanamycin, amikacin or capreomycin)severely threatens global tuberculosis control programme and pose the fear of return to an era in which drugs were no longer effective [26] .

1.1.5.1 Molecular genetic basis of drug resistance in Mycobacterium tuberculosis

The molecular genetic basis of drug resistance in *M.tuberculosis* for some anti-tuberculosis drugs is not fully known. In many cases, mutations found in association with drug-resistant tuberculosis (DR-TB) might cause different levels of resistance. Some of the mutations are not directly related to drug resistance [24]. However, molecular investigation of drug resistance in *M.tuberculosis* revealed that, resistance to basic anti-tuberculosis first-line drugs of isoniazid(INH), rifampicin(RIF), ethambutol (EMB) and streptomycin(STP) are attributed to specific mutations in target genes or regulatory domains [25].

Mutation in *KatG* gene could lead to resistance to INH. Modification of *KatG* gene (encode for catalase- peroxidase enzyme) as partial or total deletions, point mutations or insertions, leads to the abolition or diminution of catalase activity. Catalase activity is essential in activating INH to the active hydrazine derivative (isonicotinoyl). Deficiency in this enzyme activity produces high-level resistance and this is found in more than 80% of INH resistant strains [24].

In addition, *inhA* and *ahpC* genes (encode enzymes for mycolic acid biosynthesis*)* have been identified to play role in INH resistance. Point mutations in the regulatory region of these genes are found in some of INH resistant strains. About 20-34% INH resistant isolates have mutations in the promoter region of *inh*A either alone or in combination with *kat*G and 10% of INH resistant strains have been found with the mutation in the *ahp*C promoter region [24, 26].

Resistance to RIF in *M.tuberculosis* is associated with mutation in *rpoB* gene. This is the gene codes for the β-subunit of DNA dependent RNA polymerase. About the 95% RIF resistance conferring mutations in *rpoB* gene occur in a small region of 81bp. Only less than 5% of RIF resistance conferring mutation is occurring outside of this region. Molecular techniques which can identify mutations in 81bp region of the *rpoB* gene may use as rapid tests for the diagnosis of RIF resistant *M. tuberculosis* strains [24, 27]. RIF resistance related mutation in *M.tuberculosis* is rare. It occurs at a rate of 10^{-10} per cell division with an estimated frequency of 1 in 10^8 cells in drug free environment. However, during inappropriate therapy, it rapidly results in the selection of mutants that are resistant to other anti-TB drugs. Most commonly, it exists with mutation for INH resistance [28].

The major mechanism for acquisition of resistance in *M.tuberculosis* to EMB has been proposed as due to mutation in the *embB* gene. This gene encode for arabinosyl transferase enzyme. Membrane-associated arabinosyl transferase has been implicated as the targets for EMB. Arabinosyl transferase enzyme is involved in the polymerization of cell-wall arabinan of arabinogalactan and lipoarabinomannan. Inhibition of arabinan synthesis by EMB results in the accumulation of mycolic acids and leading to cell death. Alterations at codon 306 of *embB* gene have been identified as being the most common alteration in EMB-resistant *M. tuberculosis* clinical isolates. The mutation rate to EMB is 10^{-7} with one resistant strain out of 10^5 bacilli [29, 30].

The resistance of *M.*tuberculosis to STP emerges through mutations in *rrs* and *rpsL* genes. The rrs and rpsL genes are genes encode for 16S rRNA and S12 ribosomal protein respectively. The site of action of STP is the 30S subunit of the ribosome at the ribosomal protein S12 and the 16S rRNA. Resistance to STP is caused by alteration in the STP -binding site of ribosomal protein S12 and the 16S rRNA [21]. About 60% of resistance to STP has been detected in rpsL-mutated strains while 10% of resistant strains have mutations at *rrs* gene. The mutation rate for streptomycin is 10^{-8} resulting in resistance 1 out of 10^{7} bacilli [30].

1.1.5.2 Global epidemiology of drug resistance in Mycobacterium tuberculosis

The emergence of anti-tuberculosis drugs resistant *M.tuberculosis* strains is a worldwide problem. The exact global burden of DR-TB is unknown. Despite the lack of consistent data on the global burden of DR-TB, studies indicate that there is considerable global increment in DR-TB particularly of MDR-TB. WHO 2008 report of drug resistance surveillance showed that, any drug resistance among new cases was 30%. The rate of MDR-TB range from 0 to 22.3% with more than 15% in the fourteen settings [31].

The report also showed that, from sixty-six countries with separate data of drug resistance in previously treated cases, sixteen were reported more than 50% any drug resistance. The rate of MDR-TB ranges from 0 to 62.5% with the more than 25% in sixteen countries. The prevalence of drug resistance significantly high among previously treated cases than in the new cases [31].

WHO 2007 to 2010 drug susceptibility surveillance report showed that, the proportion of MDR-TB among new cases ranged from 0% to 28.9%. The figure is extremely high in previously treated cases (0% to 65.1%). WHO estimated that there were half a million MDR-TB cases in 2011. Global prevalence of MDR-TB was 3.7% and 20% in new and previously treated cases respectively. About 9% of MDR-TBs are XDR TB. In March 2013, 84 countries had reported at least one XDR-TB case [32, 33].

The higher prevalence of MDR-TB in previously treated cases is due to amplification of DR during sub-optimal drug therapy [34]. Study done in the Netherlands showed that ,out of 2901 smear –positive cases those enrolled in treatment, 373 cases were registered for retreatment, 125 failure, 80 defaulted and 168 relapse respectively. Out of 125 failure cases, 40 identical

strains were analyzed for drug resistance patterns before and after failure. About 39 of 168 relapses cases were also tested for drug resistance patterns before and after relapse[35].

Out of 40 failure cases, 15% had mono drug resistance (STP or INH), 43% had MDR-TB and 33% had poly drug resistance at the time of starting treatments. At the time of failure out of 23 cases without primary MDR-TB, 65% were acquired MDR-TB. Of 39 relapse cases 33% were susceptible, 33% resistant to single drugs, 33% resistant to other drugs and none were MDR-TB. At the relapse time, three cases with the primary resistance of INH and STP had acquired MDR-TB[35].

In low-income countries, yearly 10%–20% TB cases are registering for retreatment. In majority of these cases empirical retreatment therapy is result in unsatisfactory outcomes [36]. Study conducted in Uganda showed that, a total of 29/148 (20%) HIV-uninfected and 37/140 (26%) HIV-infected retreatment case had an unsuccessful treatment outcome. MDR- TB at enrolment was the only common risk factor for death during follow-up for both HIV-infected and HIV-uninfected cases [37].

Studies indicates that, there is a valuable variation in the rate MDR-TB among the retreatment sub-categories of relapse, defaulters and treatment failures[31].Study done in Philippines reported that out of 2438 patients who had positive cultures and tested for first -line drugs, 76% were identified as MDR-TB. MDR-TB occurred most frequently among treatment failure cases (97%) who did not demonstrate culture conversion after 3 months of treatment (91%) and who failed the treatment regimen for new cases (83%). Multiple or number of treatment is also indentified as factor for increase in MDR-TB rates. In the above study, the rates of MDR-TB were 78% and 57% for Category 2 relapse and return after default respectively. It was 33% and 22% for Category 1 relapse and return after default respectively [38].

Studies indicated that, failures for previous treatments are an indicator for MDR-TB. Study from Peru also showed that, out 173 patients identified as treatment failures on directly observed treatment short course chemotherapy(DOTS), 160(92.5%) were culture-positive TB. Of those, 150(93.8%) had MDR-TB. Sixty of the 150 (40.0%) isolates had resistance to INH, RIF, EMB and PZA while 44(29.3%) had resistance to INH, RIF, EMB, PZA and STP the first-line retreatment regimen [39].There are multiple indicators for the treatment failure.

Study done in Uganda indicates that out of 170 smear positive cases those started 2HRZE/4HR treatment regimen,60 were became smear positive at the end of five months. Positive sputum smear at 2 months of anti-tuberculosis therapy and poor adherence to anti TB treatment were predictors for treatment failure [40].

1.1.5.3 Epidemiology of drug resistant Mycobacterium tuberculosis in Ethiopia

Ethiopia is included in 27 high MDR-TB burden counties. WHO estimated that the prevalence of MDR-TB is 1.6 % in new cases and 12% in retreatment cases [41]. Due to the limited capacity to perform culture and DST, the exact national prevalence of DR-TB is unknown. However, studies indicated that, there is increasing in rate of DR-TB in retreatment cases. Study conducted in Addis Ababa showed that, 54(50%) of isolates from retreatment cases, were resistant to at least one or more drugs. Any resistance was higher in treatment failures 5/5(100%). MDR-TB was detected in 12% of the isolates[42].

Another study in Addis Ababa also showed that, out of 75 isolates from retreatment cases,44(58.7%) were resistant to at least one drug. The highest any drug resistance was found to INH (42.7%).Resistance to RIF was 33.3%. The lowest resistance was detected against EMB (9.3%). MDR-TB was observed in 21 (28%) isolate [4]. From the finding of the above two studies, it can be seen that there was more than twice elevation (12% versus 28%) in the rate of MDR –TB in within a decade [4, 42].

Report from the anti-tuberculosis drug resistance survey conducted nationwide in 2005 showed that INH mono-resistance and RIF mono-resistance, among new TB cases, was 2% and 1%, respectively. Notified prevalence of mono-resistance to INH and RIF among previously treated cases was 5.3% and 1.3% respectively. The prevalence of MDR-TB was significantly higher previously treated cases (17%) than in new cases(1.3%)[43].Moreover, WHO, MDR-TB and XDR-TB progress report 2011 showed that, there were an estimated 2000 MDR-TB cases. a total of 233(11.65%) cases notified. of these only 88(4.4%)were enrolled on treatment and no report was written on treatment outcome[44].

Recent study in Addis Ababa demonstrated high prevalence of any drug resistance (72.9%) and MDR-TB (46.3%) among retreatment cases. This was much higher than that of WHO estimates as well as reports from previous studies in the country. this study, also showed that, trend in drug resistance rate among re-treatment cases showed a significant increase for any drug, INH, RIF, and MDR-TB[45].

Furthermore, study in Jimma showed that, out of 146 isolates 5(3.4 %) were MDR –TB. Of this 3 (15.6%) were from previously treated patients. The finding showed that there was statistically significant association between previous treatment and the like hood MDR[46].

1.1.5.4 Diagnosis of Drug resistant Mycobacterium tuberculosis

Diagnosis of DR-TB strains is important requirement in management of TB. To meet this requirement many conventional culture and molecular techniques are used to assess the DR patterns of *M.tuberculosi*s isolates. Conventional culture techniques that are based on, absolute concentration, resistant ratio and proportion methods, are used to conduct DST either directly from clinical specimen or indirectly from culture isolates. However, most conversional culture techniques are based on indirect proportion method. This allows determining the proportion of resistant strains as compared to susceptible strains at pre-determined critical proportion of the drugs [2].

Conventional culture techniques for DST of *M.tuberculosi*s are previously relied on solid media.The most widely utilized solid medium for DST is egg-based Lowenstein-Jensen (LJ) medium. The use of LJ medium requires 3 to 8 weeks each for detection and identification. The DST result declaration also requires additional 3 to 8 weeks. During these time patients may remain untreated and continue to be infectious. Therefore, rapid and reliable culture techniques are important for prompt treatment and control of disease transmission [47]. Introduction of semi- automated liquid culture systems substantially reduced turnaround times in to 7 to 14 days for detection and additional 7 to 14 days for DST. In the last two decades, several liquid culture systems have been developed. From these, Mycobacteria Growth Indicator Tube (MGIT) is the wildly used liquid culture technique for DST of *M.tuberculosis* against the basic first-line drugs[48, 49].

1.1.5.4.1 The Mycobacteria Growth Indicator Tube (BACTEC MGIT / 960

The BACTEC MGIT 960 is widely used in developed countries and currently introduced in to the central and referral laboratories of developing counties [48]. The MGIT system uses the non-radioactive fluorophore for detection and DST. The system consists of a glass tube containing modified middle brook 7H9 broth base, MGIT growth supplement(OADC)and antibiotics(PANTA) that inhibit contamination[50]. Oxygen quenched fluorescent compound is embedded at the bottom of glass tube. Growth of *M.tuberculosis* in the tube result in depletion of dissolved oxygen. Following reduction in oxygen tension, the fluorescent begin to fluoresce. The fluorescent intensity can be detected manually by viewing under ultraviolet (UV) light or by a sensor built in BACTEC MGIT 960 machine. This system helps in early detection (7 to 14days) positive cases. The result declared as negative on 42 days after inoculation. Automated version is helpful for DST against first -line drugs of STP, INH, RIF, EMB and PZA at predetermined critical concentration [50, 51].

1.1.5.4.2 Molecular methods for detecting drug resistance in *Mycobacterium tuberculosis*

Advances in molecular biology tools have arisen with unprecedented opportunities for rapid detection and DST of *M.tuberculosis.* Considering the advantages of rapidity, high sensitivity and specificity, some of these methods are incorporated in to the national TB control progrmme of developed countries. Most recently, solid-phase hybridization Line Probe Assay (LiPA) and gene expert (Xpert MTB/RIF) techniques are both molecular methods used for rapid detection and DST of *M.tuberculosis*. LiPA are used for detection and DST to INH and RIF or RIF only. Gene expert (Xpert MTB/RIF) is used for detection and DST to RIF[52].

The Line Probe Assay (INNO-LiPA Rif TB Assay, Innogenetics, Ghent, Belgium) and GenoType *MTB*DR assay (Hain Life sciences, Nehren, Germany) are rapid molecular techniques used for detection and DST of *M.tuberculosis*. INNO-LiPA Rif TB assay is used for RIF resistance testing while GenoType *MTB*DR is used for MDR-TB testing [53].In both cases DNA is directly extracted from isolates or smear positive clinical specimen. The extract is amplified by PCR and PCR products are hybridized with labeled specific probes immobilized on plastic strips. Hybridization with probes is revealed by the development of a colored on the strip that allows lines to be seen where the probes are hybridized [54].

Gene expert (Xpert MTB/RIF) is an automated, cartridge-based nucleic amplification assay for detection and RIF resistance testing in less than two hours. The system integrates an automated sample processing, nucleic acid amplification, and detection of the target sequences in simple or complex samples using real-time PCR. The system consists of an instrument, personal computer, barcode scanner, and preloaded software for running tests on collected samples and viewing the results. The system requires the use of single- disposable GeneXpert cartridges that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained, cross-contamination between samples is eliminated[55].

1.1.6 Treatment of tuberculosis

The history of TB changed dramatically after the introduction of anti-tuberculosis drugs. In 1944 STP and paraaminosalicylic acid **(**PAS) were discovered as an anti-tuberculosis drugs. The combination of these drugs became the first multiple anti- tuberculosis drugs. In 1952, a third drug, INH, was added to the previous combination. INH Incorporation in to the regimen was greatly improving the efficacy of treatment. In 1960, EMB substituted PAS and in 1970s RIF was discovered and added in to the combination. Finally, in 1980, pyrazinamide (PZA) was introduced into the anti-tuberculosis treatment and the introduction of PZA is result in the reduction of treatment duration from nine months in to six months [2].

Chemotherapy of *M.tuberculosis* is fundamental for promoting the cure of the patients and breaking the chain of transmission [2]. Successful TB treatment benefits both patients and the community in which the patients reside. TB treatment with short-course multidrug chemotherapy is the cornerstone of the modern approach to control the disease [56].

WHO and international union against tuberculosis and lung disease (IUATLD) recommends first-line standard regimen comprising of INH, RIF, EMB, and PZA for new pulmonary TB patients. The combination of all these drugs is given for 2 months (intensive phase) followed by INH and RIF for 4 months as continuation phase (2HRZE/ 4HR). This regimen is also used for TB/HIV and EPTB cases with exception of bone TB and TB meningitis[57].

The first-line standard regimen for retreatment cases comprises INH, RIF, EMB, PZA and STP. The combination of these drugs is given for 2 months followed by INH, RIF, EMB and PZA for additional one month (intensive phase)[57].

Finally, the combination of INH, RIF and EMB is used for continuation phase of five months (2HRZES/1HRZE/5HRE). However, WHO recommend that national TB control programmes should obtain and use their country-specific drug resistance patterns of retreatment cases to determine the levels of MDR-TB. This can be used as a guidance for designing country or regional related retreatment regimen combination[57, 58].

In Ethiopia, five first line drugs; STP, INH , RIF, EMB and PZA are used for treatment of TB . The fixed dose combination (FDC) of these drugs for adults are available as RHZE 150/75/400/275 mg, RHZ 150/75/400 mg, RH 150/75 mg, EH 400/150mg and TB loose drugs available as EMB400 mg, INH 300 mg, STP sulphate vials 1 gm. STP is administered by injection while the others are taken orally. All the drugs preferably should be taken together as a single, daily dose[59].

Table 1. **Standard treatment regimens for adult patients in defined group in Ethiopia [59]**

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TB patient type		**Recommended regimen**	**Additional Action(s)**
New		*Treatment as new:* 2RHZE/4RH	*Send sputum for culture and DST if a contact of known MDR-TB case*
Previously treated	Treatment after failure	*Treat as retreatment:* 2RHZES/RHZE/5RHE*)*	*Send sputum for culture and DST while treating the patient*
	Treatment after default Or relapse after one course of treatment	*Treat as retreatment:* 2RHZES/RHZE/5RHE*)*	*Send sputum for culture and DST while treating the patient*
	Relapse after second or subsequent courses of treatment Failure of retreatment	Wait for DST result	*Send culture and DST and r*efer patient to MDR treatment initiating center
Transfer in		Continue same treatment regimen	*Consider DST if eligible*
Other	Defaulted patients	*Treat as retreatment:* 2RHZES/RHZE/5RHE	*Send sputum/specimen for culture and DST while treating the patient*

WHO recommendation of the 2HRZES/1HRZE/5HRE treatment regimen for retreatment cases is based on the presumption or known of drug-susceptible TB strains [60]. However, national or regional TB control programmes of resource-limited countries are commonly initiate empirical treatment of 2HRZES/1HRZE/5HRE treatment regimen for retreatment cases. Studies indicate that empirical treatment of retreatment cases with 2HRZES/1HRZE/5HRE treatment regimen without having DST is great risk for treatment failure. Moreover, empirically treated DR/MDR-TB cases show less response for this first- line treatment regimen alone and they should treated with second-line treatment regimen [57, 58, 61].

The higher prevalence of DR/ MDR-TB in retreatment cases have become a significant public health problem worldwide. In a number of countries, it becomes an obstacle to effectiveness of global TB control programme. The higher rate of drug resistant *M. tuberculosis* in retreatment cases is associated with ineffective treatment of tuberculosis, leading to acquired resistance and transmission of drug-resistant strains. The estimated global rate of MDR in retreatment cases is about 15% that is five times more than in new cases (3%) [62-64].

Studies indicated that, previously TB treated cases are account for 13% of global TB notifications. Most of these cases come up with low treatment success rate to of first- line drugs of 2HRZES/1HRZE/5HRE treatment regimen. This is due to empirical treatment without evidence of DST results in prior treatment, non-adherence to treatment, poor national TB control programme or combination of these. Moreover, the higher of MDR-TB in retreatment cases is found as the most possible reason for low unsatisfactory treatment outcome [34, 57, 65].

Therefore, establishing the level of DR among retreatment cases is important for monitoring the effectiveness of TB control programme and for selecting alternative retreatment regimen. WHO recommended DST for all retreatment cases before the initiation2HRZES/1HRZE/5HRE. National TB control programmes should obtain and use their country-specific level of DR in retreatment cases. This is important to determine setting specific prevalence of MDR-TB among these cases [65].Moreover, determining the level of DR/ MDR-TB in a particular geographic area would help in evaluating the performance of setting tuberculosis programme and in deciding the best treatment option for patients. Nevertheless, there is inadequate of information on the level drug resistance of *M.tuberculosis* complex among retreatment cases in Ethiopia.

1.2 STATEMENT OF THE PROBLEM

Tuberculosis (TB) remains a major public health crisis in sub-saharan Africa, despite declining global TB incidence rates. Achieving the United Nations Millennium Development goal to reduce the burden of TB by 50% in 2015 seems unlikely in this region. This is due to several reasons including unsuccessful treatment programme, HIV epidemic, increasing economic deprivation and the emergence of DR-TB/MDR-TB[66].

The emergence of DR-TB/MDR-TB poses a serious risk of compromising the effectiveness of TB elimination plan of 2050 (<1 case per 1 million population) [14, 18]. Resistance to anti-tuberculosis drugs has been a problem since the era of chemotherapy. However, it became recognized after dramatic outbreaks of MDR-TB in the early 1990s. Since then, nearly half a million cases of MDR-TB emerge every year. However, only 3% of these get treated and 110,000 died annually. Moreover, approximately 5 to 10% of MDR-TB cases are XDR-TB [61].

Multi-drug resistant tuberculosis (MDR-TB) requires longer treatment with less effective and more toxic second-line drugs. Drug costs for treatment of MDR-TB are considerably higher and divert resources away from managing a national TB control program of most low-income countries. The treatment outcomes for MDR-TB are poor and in some cases it can be changed in XDR-TB that responds to even fewer available ant-tuberculosis drugs [66].Moreover, XDR-TB must be treated with even more expensive and toxic third-line drugs, and a course of treatment must be specifically tailored to individual TB samples. Most patients with XDR-TB will die before such measures can be carried out in large part due to the difficulty of diagnosing the resistance in time[67].

There are also significant economic impacts of MDR-TB on patients, patient's families and communities at large. This is because; they lost much more time from work due to prolonged illness and treatment. These could impoverish patients and their families long after treatment is completed. Moreover, the high costs represent a potential barrier to completion of treatment for MDR-TB patients. WHO 2010 reports showed that, 52% of 25 000 MDR-TB cases were not completed their treatment for several reasons including, deaths (15%), treatment interruptions (14%), treatment failure (9%), and insufficient data (14%)[68].

Persistence increase of MDR-TB rates could due to high rate of transmission or acquisition of drug resistance. Thus, patients can be infected with MDR-TB from index patents as a primary drug resistance and this is more common in new MDR-TB cases. Due to an interrupted or inadequate treatment, drug susceptible *M.tuberculosis* strains can develop resistance to anti-tuberculosis drugs which known as acquired drug resistance. MDR-TB due to acquired drug resistance is mostly emerging among previously treated TB cases due mismanagement of previous TB treatment(s)[69, 70].

If patients treated; with too few drugs, for too short a period, or with the drugs to which infecting strains are partly resistant, the resistant strains are favored and will eventually predominate in the body. Hence, retreatment cases are more likely to harboring strains with full or partial drug resistance for first-line drugs in used in previous treatment. When previously treated cases are starting empirical therapy without having DST, more DR is developing for previously used drugs [36, 37, 71].

Multidrug resistant tuberculosis also poses a major challenge on treatment outcome of standard regimens for retreatment cases. Studies indicate that retreatment cases had high in-hospital mortality, or poor treatment outcome and a high rate of MDR-TB. Hence, in countries where prevalence of initial MDR-TB exceeded 3%, failure rates average was 5.0%, and relapse rates average was 12.8%, it is advisable to strengthen capacity to perform DST in treatment failure and relapse cases before the initiation of treatment [71].

WHO estimated that, in Ethiopia, the prevalence of MDR-TB among retreatment cases was 12% [44].However, this information might be old or not nationally representative. Moreover, WHO estimation of MDR tuberculosis notification are made based on either on routine surveillance (for the few countries with data), or are modeled with data from countries that are regarded as similar [67]. Thus, the prevalence MDR-TB and the size of recent increases are likely to be underestimated because of insufficient laboratory facilities for DST in the country.

Many studies have been conducted to determine the prevalence of MDR-TB either among new cases or both in new retreatment cases. In majority of these studies the numbers of retreatment cases were too few to clearly show the level of MDR-TB in this category. Relatively few studies were conducted only in particular area on separate drug resistance patterns of *M.tuberculosis*

among retreatment cases. However, finding of these studies showed that overall prevalence drug resistant *M.tuberculosis* is high among retreatment cases [4, 42].

The most recent study conducted in Addis Ababa showed that the rate MDR-TB (46.3%) is much higher than that of previous reports (28%) [4, 45].This study also pinpointed that there has been an increasing in the trend of drug resistance among the retreatment cases. Therefore, establishing the level of drug resistance in different setting is important for early case detection, designing treatment for confirmed cases and prevents transmission of drug resistant strains in the community.

RESEARCH GAP

The proportion of drug resistance is high in retreatment cases and it is independent predictor for the prevalence of MDR-TB. However, there is lack of information on drug resistance patterns of *M.tuberculosis* in previously cases in the study setting. Therefore, the aim of the study was to determine the drug resistance patterns of *M.tuberculosis* complex isolates among retreatment cases at Jimma University Specialized Hospital Southwest, Ethiopia.

1.3 SIGNIFICANCE OF THE STUDY

Establishing the level of drug resistant *M.tuberculosis* complex among previously treated TB patients is an important aspect of TB control programme. Therefore, the finding of this study will helpful in determining the level of drug resistant *M.tuberculosis* complex among previously treated cases in study setting. The finding will useful in identifying the most predominant patterns of drug resistance in M. *M.tuberculosis* complex. Moreover, the result will contribute the roles in detecting, treating and controlling of multi-drug resistant tuberculosis isolates. It will contribute role in providing direction on selecting appropriate treatment regimens for confirmed multi-drug resistant tuberculosis patients. The finding will contribute for the nationwide drug resistance surveillance in determining geographical differences in drug resistance.

The result of this study also provides information on epidemiological risk factors for drug resistance in *M.tuberculosis* complex.This information will be used for police makers, service providers and programme evaluators for developing strategies to minimize effect of potential risk factors ahead the emergence of drug resistant or multi-drug resistant tuberculosis. Moreover, the finding of this study will provide the baseline information on drug resistance patterns of *M.tuberculosis* among previously treated cases.

CHAPTER TWO

OBJECTIVES OF THE STUDY

2.1 General objective

- The objective of the study was to determine drug resistance patterns of *Mycobacterium tuberculosis* complex isolates among previously treated pulmonary tuberculosis cases.

2.2 Specific objectives

- To determine the prevalence of drug resistance in *Mycobacterium tuberculosis* complex isolates among previously treated pulmonary tuberculosis cases
- To determine the proportion of drug resistance in *Mycobacterium tuberculosis* complex isolates among sub- categories of previously treated pulmonary tuberculosis cases.
- To identify the risk factors associated with drug resistance in *Mycobacterium tuberculosis* complex isolates among previously treated pulmonary tuberculosis cases.

2.3 Hypothesis

The prevalence of multi-drug resistant tuberculosis among previously treated cases around Jimma is greater than 15.6**%**

CHAPTER THREE

METHODS AND PARTICIPANTS

3.1 Study area

The study was conducted at Jimma university specialized hospital. Jimma University specialized hospital is found in Jimma town. Jimma town is found in Jimma zone at the southwest of Ethiopia. The zone has 17 Woreda and one city administration. Based on the 2007 census conducted by the central statistical agency of Ethiopia (CSA), this zone has a total population of 2,788,390. The zone is found in an area 199316.18 km^2 and average altitude of about 2180m above sea level. It lies in the climatic zone 16% dega, 62% woyna daga and 22% kola, which considered as an ideal for agriculture as well as human settlement. The zone is generally characterized by warm climate with a mean annual maximum temperature of 33 0°C and a mean annual minimum temperature of 10°C. The zone has one district hospital, fifty-four health centers and fife hundred fifty four-health posts, fife nongovernmental fife health stations that provide health care services. The Jimma city administration has one referral hospital, one district hospital and three health centers.

Jimma university specialized hospital is teaching referral hospital. the hospital provides the medical services of internal medicine, surgery, ophthalmology, gynecology, pediatrics , pharmacy and laboratory services. The hospital has been undertaken the innovation and expansion of laboratory services. Currently the hospital laboratory provides the culture and DST for TB diagnosis and more recently, the hospital has started the GenoType *MTB*DR assay (Hain Life sciences, Nehren, Germany) are rapid molecular techniques used for detection and DST of *M.tuberculosis*

3.2 Study design and period

A prospective cross-sectional study was conducted from March 2012 to April 2013.

3.3 Study Population

3.3.1 Source population

All sputum smear positive pulmonary tuberculosis cases with history of previous treatment with ant- tuberculosis drugs.

3.3.2 Study subjects

All sputum smear positive pulmonary tuberculosis cases with previous history of treatment who were available during the data collection period.

3.3.3 Inclusion criteria

Pulmonary tuberculosis patients who were sputum smear positive for acid fast bacilli (AFB) and had the history of previous treatment for tuberculosis for at least one month. Patients who were gave their consent to include in to the study.

3.3.4 Exclusion criteria

Patients with the following characteristics were excluded from the study.

- Patients who were on anti-tuberculosis treatment for less than one month at the time of data collection
- Patients who were less than 15 years old.
- Patients those provided inadequate specimen for the laboratory analysis.
- Patients who had previous history of anti-tuberculosis treatment for less than one month.

3.3.5 Sample size

The minimum sample size required for this study was determined using the formula recommended by WHO guideline for surveillance of drug resistance, using Z value of 95% confidence interval and 2% level of precision(d=2%) as follows[82].

$$n = \frac{N z^2 p \times (1- p)}{d^2 (N- 1)^2 + p(1 - p)}$$

Where:

N = total number of previously treated smear positive TB cases registered during 2011 at Jimma zone and Jimma town. N=74

z = z-value (from the standard normal distribution) that corresponds to the desired 95% confidence interval z = 1.96)

d = level of precision (as a decimal= 0.02)

p = proportion of rifampicin resistance which is 0.156 from previous study conducted in Jimma in 2011[46].

$n = \frac{74 (1.96)^2 (0.156 \times 0.844)}{} = \mathbf{70}$

$0.02^2 \times 73 + (1.96)^2 (0.156 * 0.844)$

The sample size was increased by a factor **20 %** to compensate expected lose (patients diagnosed as smear positive who do not return to the diagnostic center or do not produce an adequate sample for the analysis ,culture contaminated or culture negative samples and samples for which DST result were not interpretable. The final sample size was **84** sputum smear positive previously treated pulmonary tuberculosis cases.

3.3.6 Patients enrollment

A total of 79 sputum smear positive previously treated pulmonary tuberculosis patients were recruited in to this study.

3.4 Study Variables

3.4.1 Dependent variables

Drug resistance patterns of *M.tuberculosis* complex isolates.

3.4.2 Independent variables

Sex ,age in years, residence of place, HIV sero status, Anti-retroviral (ART) status, retreatment sub-categories (relapse, treatment failure and defaulter) number of treatment before this episode, injection for more than one month, duration of illness for this episode, history of diabetic mellitus, cigarette smoking habit, alcohol abuse, history of being in prison, history of contact with chronically coughing individuals in the families and history of other lung disease before this episode.

3.5 Data collection

Structured and pre-tested questionnaire was used to collect socio demographic data. Information on clinical history of the patients was collected by interviewing patents and reviewing patient's medical records (annex 1).

3.5.1 Specimen collection and processing

3.5.1.1 Specimen collection

Two sputum specimens were collected in to clean, sterile, leak-proof, screw caped disposable plastic containers. The collected specimens were labelled and sealed according to standard protocol for specimen handling. From each specimen smear was prepared for microscopic examination by using Zihel Neelson staining methods. Sputum result for AFB was graded at JUMBL according to WHO/IUATLD recommendation. None…….. No AFB seen

1-9 if exact number of AFB seen per 100 fields

1+ if 10-99 AFB seen per 100 fields

2+ if 1-10 AFB seen per field

3+ if AFB seen per field were more than 10. About 3-5ml ml specimen was transferred in to 50ml graduated falcon tubes.

3.5.1.2 Specimen processing

Sputum specimens were homogenized and decontaminated by N-acetyl L-cysteine- sodium ydroxide method (NALC-NaOH) methods. Equal amount of NALC-NaOH solution was added in to the sputum specimens. Specimen - NALC-NaOH solution was filled by Phosphate- buffer solution (PBS) of 6.8 PH unit, to the level of 45ml.Then the solution was centrifuged for 15 minute at speed of 3000rpm.The supernatant was decanted and the sediment was re-suspended with 1-2ml PBS (annexIV).

3.5.1.3 Inoculation and incubation in MGIT 960 tubes

One MGIT tube per sample was labelled with sample code and date of inoculation. A volume of 0.5 ml well mixed suspension was inoculated to MGIT 960 tube containing 7.0 ml of modified middle brook 7H9 broth base that added with 0.8ml MGIT growth supplement – PANTA solution before inoculation. The barcode of all inoculated MGIT tubes were scanned and all tubes were incubated in the BACTEC MGIT 960 instrument at $37C^0$ (annex IV).

3.5.1.4 Culture reading and confirmation of growth

Culture in MGIT tubes was reported as contamination, positive or negative if instrument flagged as error, positive or negative respectively. The incubation period for contamination, positive or negative was less than 3days, 7-14 days and 42 days respectively. All positive flagged samples were confirmed for growth by visual inspection and AFB smear microscopy. Contamination was ruled out by inoculating culture suspension on blood agar for overnight incubation. Samples with AFB positive result and did not showed any growth on blood agar were considered as culture positive and were pended for identification (annex IV).

3.5.1.5 Identification of *Mycobacterium tuberculosis* complex.

Identification of *M.tuberculosis* complex was made tentatively based on growth rate, turbidity appearance and morphology of AFB. Paranitrobenzoic acid (PNB) (500µg/ml) inhibition test was used for complete identification *M.tuberculosis* complex. McFarland #0.5 standard solution comparable suspensions of positive samples were inoculated on PNB containing LJ media. The inoculated media were incubated for maximum of 28 days at 35-37C^0. If growth was not observed within 28 days, the isolates were reported as *M.tuberculosis* complex, and if growth was observed within 28 days the isolates were reported as non-tuberculosis *Mycobacteria (NTM)* (Annex IV).

3.6 Drug Susceptibility Testing (DST) against four basic first-line drugs of STP, INH, RIF and EMB (SIRE) using BACTEC MGIT 960 detection system.

Drug susceptibility testing (DST) was done on confirmed *M. tuberculosis* complex isolates using indirect proportion method. The day a MGIT tube became positive by the instrument was considered as day 0. The tube was incubated for at least one day before being used for DST in order to ruling out the contamination. A positive sample was used for DST up to day 5 after it became positive. Sample with growth greater than 5 days old was reprocessed as specimen. For samples on day 1 or day 2 undiluted culture suspensions was used for DST. For sample on Day 3, 4, or 5, 1:5 diluted suspension was used for DST (annex IV).

Suspension with 1:100 (on day 1 and 2) or 1:500(on day 3, 4 and 5) dilutions was prepared from sample or 1:5 diluted suspensions to inoculate in to growth control (GC). Five MGIT tubes per sample were labeled as GC, STP, INH, RIF and EMB. A volume of 0.8 ml of BACTEC 960

SIRE Supplement was added in to each tube. A total 0.1ml of reconstituted drugs was added in corresponding tubes labeled as STP, INH, RIF and EMB (annex).

Table-2. **Anti- tuberculosis drugs and their concentration for DST using BACTEC 960/MGIT system**

Drug	Concentration of drug after reconstitution	Volume added to MGIT tube	Final concentration in MGIT tube
STP	83 µg/ml	100 µl/ml	1 µg/ml
INH	8.3 µl/ml	100 µl/ml	0.1 µg/ml
RIF	83 µl/ml	100 µl/ml	1 µg/ml
EMB	415 µl/ml	100 µl/ml	3 µg/ml

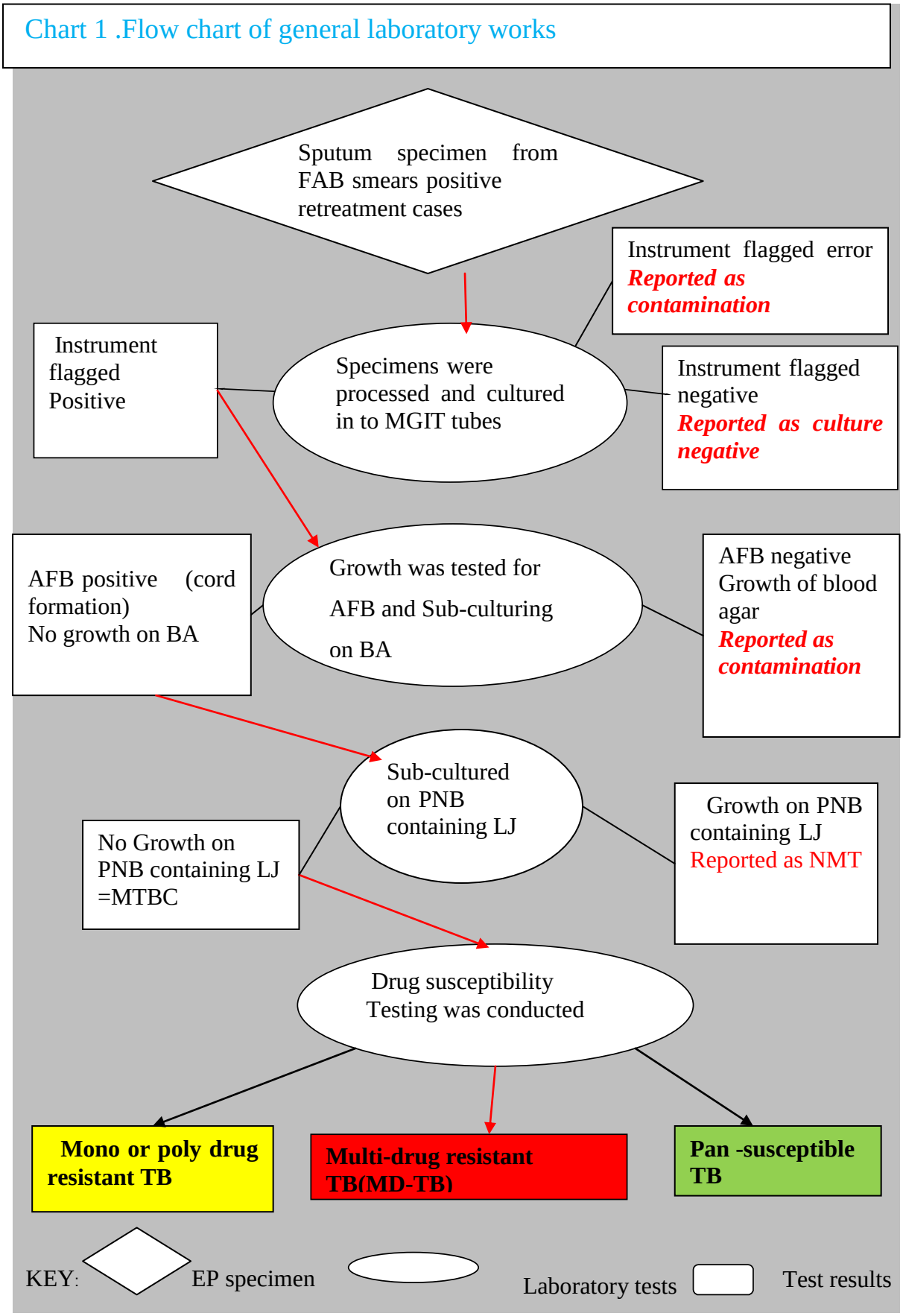
3.6.1 Inoculation of MGIT tubes for drug susceptibility Testing

A volume of 0.5 ml well-mixed 1:100/1:500 diluted suspension was inoculated into drug free tube labeled as GC. A volume of 0.5 ml sample or 1:5 diluted suspensions each was inoculated in to four drug-containing tubes labeled STP, INH, RIF and EMB. Tubes were, then placed in DST set carrier with correct sequence of GC, STR, INH, RIF, and EMB (SIRE). The set carrier barcodes were scanned and set carriers were entered into DST set entry feature of BACTEC MGIT 960 instrument (Annex IV).

3.6.2 Interpretation of results from MGIT

BACTEC 960 instrument monitors the susceptibility test set of SIRE. Once the test was complete (within 4 to 14 days), the instrument indicates that the results were ready. The susceptibility set carries were scanned and results were printed out. The result were reported as (sensitive **S**), Resistant (**R**) or indeterminate (**X**) based on printout susceptibility results from instrument (annex IV)

Chart 1 .Flow chart of general laboratory works



3.7 Data analysis and interpretation

Data were analyzed using SSPS version20. Patients 'information was cleaned and double entered into data statistical package.The distribution of drug resistance patterns of *M.tuberculosis* complex was expressed by descriptive statistics of frequency distribution tables and charts. The differences in the proportion of drug resistance patterns between groups were compared using chi–square test. Evaluation of risk factors for drug resistance of *M. tuberculosis* complex among retreatment cases were undertaken by logistic regression. For purpose of logistic regression analysis, dummy variables were used for independent variables with more than two categories. All binary categorical variables were coded as 0 and1.Finally multivariate logistic regression analysis were undertaken by including factors found significant or marginally ($P<0.25$) significant in univariate logistic analysis. $P < 0.05$ was considered as statistically significant.

3.8 Operational definition

Previously treated case: A pulmonary tuberculosis patient who has previously been treated with first line anti-tuberculosis drugs for at least one month.

New case: A pulmonary tuberculosis patient who has never had treatment for tuberculosis, or who has taken anti-tuberculosis drugs for less than one month

Treatment completed: A pulmonary tuberculosis patient who has completed treatment but who does not meet the criteria to be classified as cured or failure

Treatment failure: A pulmonary tuberculosis patient who remains smear positive , or becomes smear positive at 5^{th} month or later, while he/she is on treatment, or was smear negative at start and becomes smear positive after 2nd month of treatment.

Return after interruption (default): A pulmonary tuberculosis patient who completed at least 1 month of treatment, and returned after at least 2 months 'interruption of treatment

Relapse cases: A pulmonary tuberculosis patient who received treatment and was declared cured or treatment completed at the end of the treatment in the past but who reports back to the health service and is now found to be AFB smear-positive or culture positive

Re-infection: A recurrent disease episode with new *M.tuberculosis* strain more than 2 band change in RFLP pattern, irrespective of treatment outcome

Chronic: A pulmonary tuberculosis patient who remains smear positive after 1 treatment course completion

***First-line drugs*:** Isoniazid, rifampicin, ethambutol, pyrazinaminde and stryptomysin

***Multi- Drug Resistant tuberculosis*:** Tuberculosis caused by *M. tuberculosis* with resistance *to* at least isoniazid and rifampicin

Any drug resistance: Resistance of *M.tuberculosis* to one or more drugs, but not the combination of isoniazid and rifampicin

Extensively drugresistant tuberculosis: Tuberculosis caused by *M. tuberculosis* with resistance to at least isoniazid, rifampicin, any fluoroquinolone, and at least one of three injectable second-line drugs (amikacin, capreomycin, or kanamycin)

***Monodrug resistant tuberculosis*:** Tuberculosis caused by *M. tuberculosis* with resistance to only one of four first-line drugs

Poly drug resistant tuberculosis: Tuberculosis caused by *M. tuberculosis* with resistance to two and more drugs without the combination of isoniazid and rifampicin

***Primary resistance*:** Drug resistance of *M. tuberculosis* in a patient who has never been treated with ant-tuberculosis drugs

***Acquired resistance*:** Drug resistance of *M. tuberculosis* that occurs as a result of specific previous anti-tuberculosis treatment.

Critical concentration of drugs*:* concentration of drugs that inhibits the growth of wild type strains of *M. tuberculosis* without appreciably affecting the growth of resistant cells.

3.9 Quality assurance

To keep the quality of the data, crosschecking was conducted for consistency of information during data collection .Important variables were considered carefully to avoid any in consistency and inaccuracy of information. Concerning the laboratory data, all the activities were performed accordance with standard operating procedures. Manufacturer's instructions were followed in using MIGT media, MIGT supplements, SIRE kits and SIRE supplements. Quality of staining reagents, every new lot of MGIT media, growth supplements, SIRE kits and SIRE supplements were controlled by using standard quality control strain H37RV of *M. tuberculosis* as positive control.

3.10 Ethical consideration

Ethical clearance was secured from Jimma University, College of public health and medical sciences ethical review board. Official Letter was obtained from department of medical laboratory science and pathology. Permission was obtained from the management of Jimma University specialized hospital. Informed consent was obtained from the study participants or from the families (surrogates or guardians) of the participants before enrolling them in to the study. The objective of the study was explained to the study subjects. The possible benefits and risks of the study were described to the study participants. Confidentiality of the data was guaranteed. Any information from patients and patients' specimen was used for the purpose of the study as clearly described to them. Depending on the finding of laboratory analysis the final results were reported to the concerned physicians. MDR-TB results were communicated to the hospital TB clinic coordinator to take immediate action by linking the patients to MDR-TB treatment center.

3.11 Dissemination of results

The findings of this study will be presented to department of Medical Laboratory Science and Pathology as master thesis. The result will be disseminated to the Jimma zone health bureau, Jimma town health bureau and Jimma University Specialized Hospital clinical director office. The findings will also be disseminated to different organizations (governmental and non-governmental) those working on tuberculosis or MDR-TB prevention and control programme. Finally the findings of this study will be presented in different seminars, workshops and it also be submitted to journal for publication.

CAPTER FOUR

RESULTS

4.1. General characteristics of study population

In this study, a total of 79 previously treated sputum smear positive pulmonary tuberculosis cases were included. Out of these, 48/79 (60.8%) were males and the age range of the study population was 15 - 65 years with mean age of 32 years. Majority of the cases 67/79(84.8%) were in age range of 15 - 44 years. Most of the cases 47 /79(59.5 %) were from rural community **table 3**.

Table-3. Socio-demographic characteristic of the study participants at Jimma University Specialized Hospital, from March 2012 to April 2013

Characteristics	Frequency	Percentage (%)
Sex		
Male	48	60.8
Female	31	39.2
Total	79	100.0
Age		
15-44	67	84.8
>=45	12	15.2
Total	79	100.0
Address		
Urban	32	40.5
Rural	47	59.5
Total	79	100.0

4.2 Medical History and Clinical manifestation

The majority of the cases 77/79(97.5%) had the history of sputum smear examination and 47/79(59.5%) cases had chest x-ray examination before this episode. Ten cases, 10/79 (12.7%) had experienced injection for more than one month. Three retreatment sub-categories cases were involved in this study. Thirty-seven (46.8%) cases with relapse, 34/79 (43%) were treatment failure and 8/79(10.1%) were defaulters **table 4**.

Table 4: medical history of study participants at Jimma University Specialized Hospital, from March 2012 to April 2013

Medical history	*Frequency (no)*	*Percentage (%)*
Sputum examination before episode		
Yes	77	97.5
No	2	2.5
X-ray examination before episodes		
Yes	47	59.5
No	32	40.5
Injection for more than 1 week		
Yes	10	12.6
No	69	87.4
Number of Treatment		
One time	43	54.4
Two or more times	36	45.6
Retreatment sub-categories		
Relapse	37	46.8
Defaulters	8	10.2
Treatment failure	34	43.0

The clinical finding of the study participants indicates that, all of the cases had fever and productive cough for more than two weeks. Almost all 77/79 (97.5 %) cases said that they had unintentional weight loss. Majority of the cases 75/79(94.9%) and 73/79(92.4%) were complained for tiredness and night sweats respectively **table 5.**

Table 5: Clinical manifestation of the study participants at Jimma University Specialized Hospital, from March 2012 to April 2013

. Clinical manifestation	Frequency	Percentage (%)
Cough more than 2 wks		
Yes	79	100.0
No	0	0%
Fever		
Yes	79	100.0
No	0	0%
Unintentional Weight loss		
Yes	77	97.5
No	2	2.5
Total	79	100.0
Tiredness		
Yes	75	94.9
No	4	5.1
Total	79	100.0
Chest pain		
Yes	71	89.9
No	8	10.1
Total	79	100.0
Shortness of breathing		
Yes	65	82.3
No	14	17.7
Total	79	100.0
Night sweat		
Yes	73	92.4
No	6	7.6
Total	79	100.0
Haemoptysis		
Yes	28	35.4
No	51	64.6
Total	79	100.0

4.3 Perceived predictors for tuberculosis

The HIV sero status result was recorded for 74/79(93.7%) of the cases. Of these HIV was detected in 23/79(29.1%) of the study participants. Majority of HIV positive cases 16/23(69.6%) were on ART. The result of 5/79(6.3%) cases was unknown. Majority of the HIV positive cases 21/23(91.3%) were in age groups between 15and 44 years. Twenty-four (30.4%) cases had the history of other lung disease before this episode **Table 6**.

Table 6.**The distribution of perceived predictors for tuberculosis in study participants at Jimma University Specialized Hospital, from March 2012 to April 2013**

Risk factors	***Frequency***	***Percentage***
HIV sero-status		
Positive	23	29.1
Negative	51	64.6
Unknown	5	6.3
Total	79	100
History of being in prison		
Yes	14	17.7
No	65	82.3
Total	79	100
Cigarette smoking habit		
Yes	11	13.9
No	68	86.1
Total	79	100
Alcohol abuse		
Yes	10	12.7
No	69	87.3
Total	79	100
Contact with chronically coughing individual in the families		
Yes	10	12.7
No	69	87.3
Total	79	100
Diabetic mellitus		
Yes	6	7.6
No	73	92.4
Total	79	100
History of other lung disease		
Yes	25	31.6
No	54	68.4
Total	79	100

4.4 Culture and Identification

Out of the 79 sputum specimens processed for culture 71/79 (89.9%) were culture positive and 8 /79(10.1%) were culture negative. Almost all 70/71 (98.6%) isolates were sensitive to PNB and identified as *M.tuberculosis* complex. One (1.4%) isolate was resistant to PNB and identified as *mycobacteria* other than tuberculosis.

4.5 Drug susceptibility testing against first line drugs

Drug susceptibly testing was done for all (70) culture positive isolates. Of these, 61/70 (87.1%) were from patients in the age range of 15 - 44 years and 45/70(64.3%) were from males. These isolates were from three retreatment sub-categories: 33/70(47.1%) isolates from relapse cases 29/70(41.4%) from treatment failures and 8/70(11.4%) from defaulters. Twenty-nine (41.4%) isolates were susceptible to all four first -line anti-tuberculosis drugs (STP, INH, RIF and EMB).A total of 41/70(58.6 %) isolates were resistance to at least one drug. The highest proportion of any resistance was observed to INH 36/70(51.4%). Any resistance to STP was 30/70(42.9%). Twenty-three (32.9%) of the 70 isolates were resistant RIF. Relatively low resistance was observed to EMB 20/70(28.6%). (**figure 1**).

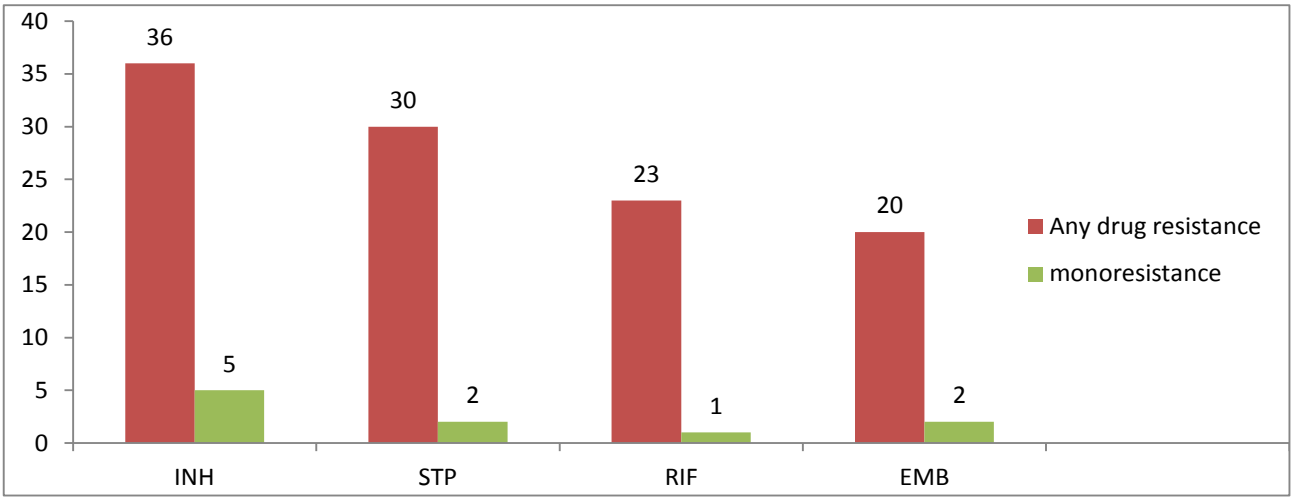


Figure1. Drug resistance patterns of *M. tuberculosis* complex isolates to each of the five first-line anti- tuberculosis among study participants at Jimma University Specialized Hospital, from March 2012 to April 2013

In this study, 22/70 isolates were resistant to at least INH and RIF, which resulted in a 31.4 % prevalence of MDR-TB among previously treated cases. From these, 15/22(68.2%) isolates were from males and 20/22(90.9%) were from cases in the age range between of 15 – 44 years. Majority of MDR-TB isolates 16/22(72.7%) were from cases with the history of treatment failures (**Table 7).**

Table 7: The distribution of MDR-TB isolates in sex, age and retreatment sub-categories from retreatment cases at Jimma University Specialized Hospital, from March 2012 to April 2013

	MDR-TB	
Characteristics	**Yes (%)**	**No (%)**
Sex		
Male	15(68.2)	30(62.5)
Female	7(31.8)	18(37.5)
Total	**22**	**48(100)**
Age in years		
15-44	20(90.9)	41(85.4)
≥45	2(9.1)	7(14.6)
Total	**22(100)**	**48(100)**
Retreatment sub-categories		
Relapse	6(27.3)	27(56.3)
Treatment failure and defaulter	16(72.7)	21(43.7)
	22(100)	**48(100)**

All 22 MDR –TB isolates were demonstrated resistance to other first-line drugs. Majority of MDR-TB isolates 12/22(54.5%) were resistant to all four first-line drugs. In our study, mono and poly drug resistance were detected in 10/70(14.3%) and 9/70(12.9%) respectively.The highest rate of mono drug resistance was observed against INH 5/70(7.1%). There was only 1/70 (1.4%) RIF nonresistant isolate. Resistance to two or more drugs (including poly and multi drug

resistance) were observed in 31/70(44.3 %). Out of these 7/31(22.6%) isolates were resistant to two drugs whereas (24/31(77 %) were resistance to three and more than three drugs **(Table 8).**

Table 8: Pattern of drug resistance in *M.tuberculosis c*omplex isolates from retreatment cases at Jimma University specialized Hospital from March 2012 to April 2013(n=70)

Drug resistance patterns	*Frequency (no)*	*Percentage (%)*
Any drug resistance	41	58.6
INH[1]	36	51.4
RIF[2]	23	32.9
STP[3]	30	42.9
EMB[4]	20	28.6
Two drugs resistance		
INH+STP	4	5.7
INH+EMB	2	2.9
STP+EMB	1	1.4
Three or more drugs resistance		
INH+RIF+STP	9	12.9
INH+RIF+EMB	1	1.4
INH+ STP+EMB	2	2.9
INH+RIF+STP+ EMB	12	17.1
MDR-TB[5]	22	31.4
Poly drug resistance [6]	9	12.9
Mono drug resistance [7]	10	14.3

INH[1] = Isoniazid, RIF[2] = Rifampicin, STP[3],= Streptomycin , EMB[4]= Ethambutol, MDR[5] = Multi drug resistance (resistance at least INH and RIF), Poly drug [6]= resistance to two and more drugs without the combination of INH and RIF , monodrug [7] =single drug resistance

4.6 Risk of drug resistance in *Mycobacterium tuberculosis*

Totally, 41/70(58.6%) strains were resistant to at least one more the four first-line anti-tuberculosis drugs. Univariate analysis was performed to identify factors associated with any drug resistance in the *M.tuberculosis* isolates. The results of analysis showed that residence place, number of treatment and duration of illness had impact on the development of any drug resistance**Table-9.**

Table 9: Univariate analysis of risk factors for any drug resistance in *M.tuberculosis* complex isolates from retreatment cases at Jimma University specialized Hospital from March 2012 to April 2013(n=70)

	Any drug resistance			
Variables	**Yes**	**No**	**COR*(95%CI)**	**p-value**
Sex				
Male	27(65.9%)	18(62.1)	1.18(0.438,3.171)	0.745
Female	14(34.1%)	11(37.9%)	1	
Age in years				
15-44	37(90.2%)	24(82.8%)	1.93(0.47,7.91)	0.474**
≥ 45	4(9.8)	5(17.2%)	1	
Residence place				
Urban	22(53.7%)	10(34.5%)	2.3(1.83, 5.87)	0.015
Rural	19(46.3%)	19(65.5%)		
HIV sero-status				
Positive	15(36.6%)	8(27.6)	.66(0.24,1.86)	0.431
Negative	26(63.4%)	21(72.4%)	1	
ART status				
Currently on ART	10(66.7%)	6(75 %)	0.67(0.97,4.58)	0.680**
Currently not on ART	5(33.3%)	2(25%)	1	

Retreatment sub-categories				
Relapse	20(48.8%)	13(44.8)	1	0.744
Treatment failure and defaulters	21(51.2%)	16(55.2%)	0.83(0.33,2.22)	
Number of treatment before this episode				
One time	19(46.3%)	19(65.5%)	1	0.021
Two and more	22(53.7%)	10(34.5%)	2.2(1.72,5.89)	
Injection for more than month				
Yes	5(12.2%)	5(17.2%)	0. 67(0.17,2.55)	0.731**
No	36(87.8%)	19(82.8%)	1	
Duration of illness for this episode				
≤2 months	11(26.8%)	13(44.8%)	1	0.016
≥ three months	30(73.2%)	16(55.2%)	2.26(1.81,6.10)	
Diabetic mellitus				
Yes	3(6.9%)	2(6.9%)	0.94(0.15-6.00)	1.000**
No	38(92.7%)	27(93.1%)	1	
Cigarette smoking				
Yes	6(14.6%)	3(10.3%)	1.49 (0.34,6.50)	0.726**
No	35(85.4%)	26(89.7%)	1	
Alcohol abuse				
Yes	6(14.6%)	3(10.3%)	0.67(0.15,2.95)	.726**
No	35(85.4%)	26(89.7%)	1	
History of being prison				
Yes	8(19.5%)	4(13.8%)	66(0.18,2.44)	0.749**
No	33(80.5%)	25(86.2%)	1	
Chronically coughing individual in the families				
Yes	3(7.3%)	5(17.2%)	2.64(0.58,12.10)	0.262**
No	38(92.7%)	24(82.8%)		
History of other lung diseases				
Yes	15(36.6%)	7(24.1%)	0.55(0.19,1.56)	0.272
No	26(63.4%)	22(75.9%)	1	

*Crude odds ratio ** Fisher's Exact Test p-value

In multivariate logistic regression analysis, residence place became the strongest association with any drug resistance. Patients from urban community were 3.44 times (95%CI: 1.12 to 10.60; p=0.032) more likely to have any drug resistance than patients from rural community. Other influencing factors included long duration of illness [OR 3.00 (95%CI: 1.17 to 10.69; p=0.039] and number of treatment before this episode [OR 2.99 (95%CI: 1.01to 8.86; p=0.048)]**table-10**.

Table-10: Multivariate analysis of risk factors for any drug resistance in *M.tuberculosis* complex isolates from retreatment cases at Jimma University specialized Hospital from March 2012 to April 2013(n=70)

	Any drug resistance			
Variables				
	COR*(95%CI)	**p-value**	**AOR" (95%CI)**	**p-value**
Residence place				
Urban	2.2(0.83 ,5.87)	0.115	3.44(1.12,10.60)	0.032
Rural	1			
Duration of illness				
≤ 2 months	1	0.121	3.00(1.17,10.69)	0.039
≥ 3 months	2.2(0.8,6.10)			
Number of treatment before this episode				
One time	1	0.115	2.99(1.01,8.86)	0.048
Two or more time	2.2(0.83 ,5.87)			

Twenty-three (32.9 %) of the 70 isolates were resistant to RIF. Univariate analysis demonstrated that a there was no significant association between RIF resistance with gender, age and residence place. The relative higher likelihood of RIF resistance was demonstrated with HIV infection, retreatment sub-categories, alcohol abuse and history of being in prison **table -11**.

Table 11: Univariate analysis of risk factors for RIF resistance in *M.tuberculosis* complex isolates from retreatment cases at Jimma University specialized Hospital from March 2012 to April 2013(n=70)

	Rifampicin resistance			
Variables	**Yes**	**No**	**COR*(95%CI)**	**p-value**
Sex				
Male	16(69.6%)	29(61.7%)	1.42(0.49,4.12)	0.520
Female	7(30.4%)	18(38.3%)	1	
Age in years				
15-44	21(91.3%)	40(85.1%)	1.84(0.35,9.64)	0.708**
≥ 45	2(8.7%)	7(14.9%)	1	
Residence place				
Urban	12(52.2%)	20(42.6%)	1.47(o.54,4.01)	0.449
Rural	11(47.8%)	27(57.4%)	1	
HIV sero-status				
Positive	11(50%)	12(27.9%)	2.67(0.937,7.63)	0.066
Negative	11(50%)	33(72.1%)	1	
ART status				
Currently on ART	8(72.7%)	8(66.7%)	1.33(0.22,7.98)	1.000**
Currently not on ART	3(27.3%)	4(33.3%)	1	
Retreatment sub-categories				
Relapse	7(30.4%)	26(55.3%)	1	0.054
Treatment failure and defaulters	16(69.6%)	21(44.7%)	2.8(0.98,8.15)	
Number of treatment before this episode				
One time	13(56.5%)	25(53.2%)	1.14(0.42,3.12)	0.793
Two and more	10(43.5%)	22(46.8%)		

Injection for more than month				
Yes	2(8.7%)	8(17%)	0.46(0.90,2.39)	0.480**
No	21(91.3%)	39(83%)	1	
Duration of illness for this episode				
≤2 months	6(26.1%)	18(38.3%)	1	0.315
≥ three months	17(73.9%)	29(61.7%)	1.76(0.59,5.29)	
Diabetic mellitus				
Yes	2(8.7%)	3(6.4%)	1.4(0.22,9.00)	1.000**
No	21(91.3%)	44(93.6%)	1	
Cigarette smoking				
Yes	3(13%)	6(12.8%)	1.03(0.23,4.53)	1.000**
No	20(87%)	41(87.2%)	1	
Alcohol abuse				
Yes	5(21.7%)	4(8.5%)	2.99(0.72,12.42)	0.143
No	18(78.3%)	43(91.5%)	1	
History of being in prison				
Yes	6(26.1%)	6(12.8%)	2.41(0.68,5.55)	0.189**
No	23(73.9%)	47(87.2%)	1	
Chronically coughing individual in the families				
Yes	3(13%)	5(10.6%)	1.26(0.27,5.80)	1.000**
No	20(87%)	42(89.4%)	1	
History of other lung disease				
Yes	8(34.8%)	14(28.9%)	1.26(0.44,3.64)	0.673
No	15(65.2%)	33(70.2%)		

On multivariate regression analysis of the data, retreatment sub-categories was independently associated with RIF resistance. Patients with the history of treatment failures were 3 times (95%CI: 1.02to 9.10; p=0.047) more likely to develop RIF resistance than relapse cases. Infection with HIV was also marginally associated with RIF resistance. HIV positive cases were 3.89 time more likely to develop RIF resistance as compared to HIV negative cases (95%CI: 0.97to 8.64;p= 0.057) **table 12.**

Table 12: Multivariate analysis of risk factors for rifampicin resistance in *M.tuberculosis* complex isolates from retreatment cases at Jimma University specialized Hospital from March 2012 to April 2013(n=70)

	Rifampcin Resistance			
Variables				
	COR*(95%CI)	**p-value**	**AOR" (95%CI)**	**p-value**
HIV sero-status				
Positive	2.67(0.937,7.63)	0.066	2.89(0.97,8.64)	0.057
Negative	1		1	
Retreatment sub-categories				
Relapse	1	0.054	1	0.047
Treatment failure	2.8(0.98,8.15		3.04(1.02,9.10)	
Alcohol abuse				
Yes	2.99(0.72,12.42)	0.143	2.89(0.61,13.58)	0.180
No	1		1	
History of being in prison				
Yes	2.41(0.68,5.55)	0.189	1.80(0.43,7.57)	0.421
No	1		1	

Multi-drug resistance (MDR-TB) was detected in 22/70(31.4 %) of isolates. Univariate analysis of the data indicated that patients with history of treatment failures were at a higher risk of acquiring MDR-TB isolates compared to relapse cases. Other actors included HIV sero –status, alcohol abuse and history of being in prison were seems to have impact on the development of MDR-TB **table-13.**

Table 13: Univariate analysis of risk factors for MDR-TB in *M.tuberculosis* complex isolates from retreatment cases at Jimma University specialized Hospital from March 2012 to April 2013(n=70)

Variables	MDR-TB			
	Yes	**No**	**COR*(95%CI)**	**p-value**
Sex				
Male	15(68.2%)	30(62.5%)	1.27(0.44,3.75)	0.645
Female	7(31.8%)	`18(37.5%)	1	
Age in years				
15-44	20(90.2%)	41(85.4%)	1.71(0.32,8.98)	0.709**
≥ 45	2(9.1%)	7(14.6%)	1	
Residence place				
Urban	12(54.5%)	20(41.7%)	1.68(0.61,4.64)	0.317
Rural	10(45.5%)	28(58.3%)	1	
HIV sero-status				
Positive	10(47.6%)	13(29.5%)	2.24(0.78,6.43)	0.133
Negative	11(52.4%)	31(70.5%)	1	
ART status				
Currently on ART	8(80%)	8(61.5%)	2.5(0.37,16.89)	0.405**
Currently not on ART	2(20%)	5(38.5%)	1	
Retreatment sub-categories				
Relapse	6(29.3%)	27(56.2.%)	1	0.028
Treatment failure and defaulter	16(72.7%)	21(43.8%)	3.43(1.14,10.28)	
Number of treatment before this episode				
One time	12(54.5%)	26(54.2%)	1	0.976
Two and more	10(45.5%)	22(45.8%)	1.02(0.37,2.80)	
Injection for more than month				

Yes	2(9.1%)	8(16.7%)	1	0.488**
No	20(90.9%)	40(83.3%)	2.00(0.30,10.31)	
Duration of illness for this episode				
≤2 months	6(27.3%)	18(37.5%)	1	0.405
≥ three months	16(72.7%)	30(65.2%)	1.6(0.53,4.83	
Diabetic mellitus				
Yes	2(9.1%)	3(6.2%)	1.5(0.23,9.69)	O.646**
No	20(90.9%)	45(93.8%)	1	
Cigarette smoking				
Yes	3(13.6%)	6(12.5%)	1.11(0.25,4.90)	1.000**
No	19(86.4%)	42(87.5%)	1	
Alcohol abuse				
Yes	5(22.7%)	4(8.3%)	3.24(0.78,13.51)	0.128**
No	17(77.3%)	44(91.7%)	1	
History of being in prison				
Yes	6(27.3%)	6(12.5%)	2.63(0.74,9.34)	0.174**
No	16(72.7%)	42(87.5%)		
Chronically coughing individual in the families				
Yes	3(13.6%)	5(10.4%)	1.36(0.30,6.27)	0,488**
No	19(86.4%)	43(89.6%)		
History of Other lung disease				
Yes	7(31.8%)	15(31.8%)	1.03(0.35,3.04)	0.962
No	15(68.2%)	33(68.8%)	1	

In multivariate logistic regression analysis, retreatment sub-category was the only strongly associated factor with MDR-TB. Patients with the history of treatment failures were 3.43 time (95% CI: 1.14to 10.27; p=0.0280 more likely to have MDR-TB than the relapse cases **Table- 14**

Table 14: Multivariate analysis of risk factors for MDR-TB in *M.tuberculosis* complex isolates from retreatment cases at Jimma University specialized Hospital from March 2012 to April 2013(n=70)

	MDR-TB			
Variables				
	COR*(95%CI)	**p-value**	**AOR'' (95%CI)**	**p-value**
HIV sero-status				
Positive	2.24(0.78,6.43)	0.133	2.30(0.74,7.14)	0.151
Negative	1	1		
Retreatment sub-categories				
Relapse	1	0.028	1	0.028
Treatment failure	3.43(1.14,10.28		3.43(1.14,10.28)	
Alcohol abuse				
Yes	3.24(0.78,13.51)	0.128	3.47(0.78,15.63)	0.106
No	1		1	
History of being in prison				
Yes	2.63(0.74,9.34)	0.174	1.2(0.46,8.36)	0.359
No	1		1	

CHAPTER FIVE

DISCUSSION

In this study the socio- demographic profiles of participants indicates high number of re-treatment cases were males (60.8%). Majority of the cases (84.8%) belong to age group 15 to 44 years. This is consistent with the study conducted in Addis Ababa where males constitute 62.5 % and 87% cases in the age between 15 and 49 [42]. The higher prevalence of TB in males might be due to differences in the societal roles of men and women, variation in risk of exposure and/or gender differences in access to health care [62].

Information on medical history of study participants were assessed in this study. Most common reason for retreatment was relapse (46.8%). This is in agreement with studies from Addis Ababa and Uganda [34, 72]. The highest recurrence or relapse rate is an indicator for previous treatment interruption and/or failure. However, the relapse may be due to endogenous reactivation or exogenous re-infection. Thus, molecular studies are recommended to exclude the impact of exogenous re-infection. Majority of the cases had the history of sputum examination in previous episode (97.5%). From this, it could be estimated that some of them might be smear positive cases in previous episodes

Majority of cases were showing typical clinical manifestation of TB during this episode. All cases (100%) were complaining for productive cough for more than 2 weeks. This is comparable with the study conducted in Zimbabwe (95.5%) [73].and Addis Ababa (100%)[4]. This can indicate the importance of clinical screening for respiratory symptoms for cases detection. Study participants were also assessed for perceived predictors of TB. Twenty-five (31.6%) cases respond that they had the history of lung disease before this episode and one-third (29.1%) cases were co-infected with HIV and many of these (69.5%) on anti-retroviral therapy.

Drug resistant *M.tuberculosis* strains have a great public health importance. Patients infected with DR/MDR-TB strains are poorly response to the first-line anti-tuberculosis drugs. Hence curing these cases is the most challenging task for TB control programme. Treatment outcomes of DR/MDR-TB using second-line drug tend to be poor, drugs are very costly and it require long duration within specialized center [69, 74].

In this study, the overall resistance to one or more drug(s)was 58.6%.This was in agreement with the previous study in Addis Ababa in which 58% of isolates were resistant to one or more drugs[4]. However, Any drug resistance rate in the present study was lower than the rate observed in recent study in Addis Ababa where 72.9 % isolates were resistant to one or more drugs [45]. This difference in findings might be due to difference in proportion of retreatment sub-categories since majority of the cases in that study were referral cases and most referral cases were treatment failure.

Residence place, duration of illness and number of treatments before this episode were showed significant association with any drug resistance. The current national TB guideline recommends the extended treatment (2RHZES/RHZE/5RHE) for previously treated TB patients by simply adding STP to the regimen. This repeated treatment practice can amplify resistance in these patients who are likely to have developed resistance to some or all of the previously used first line anti-tuberculosis drugs [61]. The situation is further complicated in developing countries where there is lack of the laboratories that have capacity for culture and DST. This challenge could be surmounted by giving priority for previously treated TB cases for culture and DST.

The highest proportion of any drug resistance was observed to INH (51.4%).This is comparable with the study done in India (52%)[22] and recent report from study in Addis Ababa (56.1%) [45]. However, our finding was higher than that of previous studies in Ethiopia 42.7%[42] and 44%)[4]. The higher proportion of INH resistance as compared to previous studies could be due to change in the prevalence of INH resistance with time.

The higher prevalence of INH resistance has also important implications. INH is the cornerstone drug used throughout the course of non-MDR-TB treatment. It is also the drug of choice for chemoprophylaxis of TB in developing countries for treating latent TB infection. Loss of the effectiveness of this drug compromises both the preventive therapy and treatment of TB disease[75]. Moreover, it is predictor for probability of MDR-TB in the future since MDR-TB often develops from initial INH monoresistant strains[76] .

The second highest any resistance was resistance to STP (42.9%).The figure is high when compared with earlier studies in Ethiopia in 1998 and 2003; where STP resistances accounted for 21% and 28% respectively [4, 42].

However, the result is lower than that of recent study in Addis Ababa (67.3%)[45].The highest any resistance to STP may due to its early introduction , its common use for treatment of other bacterial infections and inadequate treatment due to poor compliance by patients [24]. Furthermore, higher STP resistance in this study can contribute for actively working on decision of limiting STP use in the second-line treatment regimen for MDR-TB strains.

The rate of RIF resistance was (32.9%). This is in agreement with the study in Addis Ababa (33.3%)[4].The higher rate of RIF resistance might be due to its adverse effects such as nausea, vomiting, rashes, hepatitis, GIT upset, flu-like symptoms, fever and jaundice, which could result in patient non-adherence and hence may lead to the selection of resistant strain [76]. The implications of this finding would support the restriction of RIF use for non-*mycobacteria* treatment, to protect the efficacy of this drug.

In this study, there was only one case with RIF monoresistance and this is higher than the report from study in Addis Ababa in which there was no RIF monoresistance[45].The low proportion (1.4%) of non-MDR RIF resistance in this study would support the evidence of RIF resistance used as a surrogate marker for MDR-TB. It is also in line with WHO recommendation of non MDR-TB RIF resistance (<3%) as good quality performance indicator [31].

The proportion of EMB any resistance was 28.6%. EMB is the first-line drug included in the regimen of second-line drugs to treat MDR-TB in many countries including Ethiopia[16].It enhances the effect of many drugs including beta lactams, against different *Mycobacteria* species and hence used to develop a regimen for MDR-TB [76]. However, high rate of EMB resistance would challenge its inclusion in MDR-TB therapy as this may leads to unintentional incorrect therapy. Thus, further study is recommended to know the level of EMB resistance specifically in MDR-TB isolates. This can help in developing national or regional standardized second-line treatment regimen for MDR-TB cases [77].

Our study finding showed high prevalence of MDR-TB (31.4%) which is higher than WHO estimates in Ethiopia (12%)[1].The figure was also higher than previous study in Addis Ababa (28%) [4] and study in Uganda (17.8%)[34].Our finding is lower than the recent report from Addis Ababa (46.3%)[45] India (47.1%)[22] and Philippines(76.4%)[38] .The discrepancy in findings between the present study and that of recent study in Addis Ababa can be explained by

the differences in the nature of the populations included in studies. Study in Addis Ababa was conducted retrospectively on data from national TB diagnosis and treatment centre. Most of the cases were referral cases. Most of the referral cases for DST and culture in Ethiopia are treatment failures. However, our study included both referral patients and smear positive previously treated cases diagnosed at Jimma University specialized hospital. In addition there may also be geographical variation in the level of drug resistance.

In the present study, there were higher proportions of males (62.8%) with MDR-TB than females. This is consistent with a report from study in Addis Ababa [45].Majority of MDR-TB cases were in the age between 15 and 44(90.9%). This is in agreement with the report from Iran[78].The high frequency of MDR-TB among a young age group may indicate the likelihood of propagation of MDR-TB in the community because of high mobility of youth from place to place. This also suggests the occurrence of recent transmission of TB infection because the rate of TB in the older age group mostly suggests the infection has been acquired in the past [78].

The emergence of MDR-TB is exclusively due to chromosomal alterations such as mutations or deletions. These chromosomal alterations affect either the drug target or bacterial enzymes activating/modifying the drug. Beside to this, TB service related factors have a significant impact on the emergence and transmission MDR-TB[79, 80]. In this study, HIV sero status, alcohol abuse and history of being in prison were seems to have impact of MDR-TB but not significantly associated. The history of category I and category II treatment failures were identified as the strongest predictor for MDR-TB. This is consistent with previous studies in Addis Ababa [4]and study done in china [79]. Our result showed that more than 50% of treatment failures were identified as MDR-TB. Majority of these cases were from category I treatment failures (75.6%). This indicates the importance of early requesting for culture and DST than awaiting the outcome of extended category II among patients for whom category I failed.

High rates of MDR-TB among treatment failures (72.7%) can be influenced by the acquisition of resistance in the intensive and continuation phases of treatment or the rate of primary MDR-TB infection [81]. However, the relative rate of MDR-TB among newly diagnosed TB patients in Jimma was low (1.5%) [46]. Therefore, the most possible reason for higher rate of MDR-TB in our study is acquisition of drug resistance during the intensive or/and continuation phases of

treatment. This may provide clue for the importance of evaluation of currently available TB control programs on proper usage of the drugs. Moreover, it will support the necessity of looking in to the adherence of patients to full course of chemotherapy.

This study has its own limitations. It is a hospital-based study and hence there could have been significant referral bias involved in patient selection. Since DST is not recorded in the previous treatments we were unable to determine the extent of amplification in the acquired drug resistance in study population. Data about people's contact with DR-TB or MDR-TB were not available. Despite this limitation, this study provides important information on drug resistance among previously treated cases in the study setting. This can be used for better planning of TB management in regions for tackling further increasing in the level of MDR-TB and control is transmission in the communities.

5.1 Conclusion and Recommendation

5.1.1 Conclusion

The present study demonstrated higher prevalence of any drug resistance among previously treated cases around Jimma. There was a higher prevalence of MDR-TB as compared to the WHO estimates for Ethiopia. The proportion of MDR-TB was significantly higher among patients with the history treatment failures as compared to relapse cases. This has some important implications. Firstly, high prevalence of MDR-TB in study setting may pose the question on the in effectiveness of treatment for drug-susceptible *M.tuberculosis* strains. Secondly, higher proportion of MDR-TB among patients with history of treatment failures implies the importance of early identification and referring of treatment failures for culture and drug susceptibility testing. Furthermore, the importance of prompt initiating of second-line treatments for confirmed MDR-TB cases is unquestionable.

The rate of MDR-TB was also high in productive and most mobile age group. This reveals the likely hood of its impact on either patient's families or community in which patients resided. The highest any resistance was found to INH (51.4%). This indicates the probability increasing in the prevalence of MDR-TB in the future. Almost all (95.7%) of RIF resistant isolates were MDR-TB and this support the evidence of using RIF resistance as remarks for MDR-TB.

5.1.2 Recommendation

National or setting tuberculosis control programme should actively work on strict supervision of patients compliance to full course of chemotherapy and on strengthening the DOTs strategies. Increasing in community awareness of the problem of drug resistant tuberculosis particularly MDR-TB should be planed and implemented in to practice. TB patients with history of treatment failures should timely be identified and referred for culture and drug susceptibility testing. Tuberculosis control programme should also be work on early detection, treatment and control of multi-drug resistant tuberculosis. Rapid drug resistance testing techniques particularly for INH and RIF or RIF should be introduced in the study setting. Collaborative activities of the government, communities and partner organizations should be established for provision of special care for confirmed MDR-TB cases. Control of MDR- TB at entry points in collaboration with all the concerned bodies and providing useful input for future policies to should be prioritized. Strict follow up of close contact with confirmed MDR-TB cases is also an area of intervened.

Furthermore, studies should be conducted on differentiation of MTBC to species level and looking for drug resistance patterns variation among different species. In addition close monitoring of transmission trends of MDR-TB should be considered through increasing awareness of both health care workers and researches. Multicenter surveillance on the prevalence and trends of MDR-TB should be conducted. Moreover, furthers studies should be conducted in order to identify bacterial, environmental and host related determinants on both the (emergence (development) and transmission of MDR-TB at individual and population levels.

REFERENCES

1. Palomino JC, Leão SC, Ritacco V. Tuberculosis 2007,from basic science to patient care. Available at: www.TuberculosisTextbook.com. Accessed 12 September 2010.
2. McMurray N. Mycobacteria and Nocardia.In: Baron S. (eds).Medical Microbiology. Galveston: University of Texas Medical Branch at Galveston, 2000,1172-1188.
3. Meskel DW, Abate G, Lakew M, Goshu S, Aseffa A. Anti-tuberculosis drug resistance among retreatment patients seen at St Peter Tuberculosis Specialized Hospital. *Ethiop Med J* 2008;46(3):219-25.
4. Jensen PA, Lambert LA, Iademarco FM, Ridzon R. Guidelines for preventing the transmission of *Mycobacterium tuberculosis* in health-care settings. *MMWR*2005; 54(17);1-141
5. Transmission-and-pathogenesis-of-tuberculosis.-Available-at: www.cdc.gov/**tb**/education/corecurr/pdf/chapter2.pdf. Accessed 24 January 2011.
6. Ahmad S. Pathogenesis, immunology and diagnosis of latent *Mycobacterium tuberculosis* infection. *Clin. Dev. Immunol* 2011:17.
7. Centers for Disease Control and Prevention(CDC). National center for HIV/AIDS, Viral hepatitis, STD, and TB prevention- Division of tuberculosis elimination. The difference between latent TB infection and TB disease. Available at: http://www.cdc.gov/tb/publications/factsheets/general/LTBIandActiveTBpdf. Accessed 12 October 2011.
8. Paton NI, Chua YK, Earnest A, Chee CB. Randomized controlled trial of nutritional supplementation in patients with newly diagnosed tuberculosis and wasting. *Am J Clin Nutr*2004 80:460 -5.
9. Ddungu H, Johnson JL, Smieja M, Mayanja-Kizza H. Digital clubbing in tuberculosis relationship to HIV infection extent of disease and hypoalbuminemia. *BMC Infect Dis* 2006;6:45.
10. World Health Organization (WHO).Global tuberculosis Control 2010 report. Available at: http://www.who.int/tb/publications/global_report/2010/en/.Accessed 6 May 2011.
11. World Health Organization (WHO). Global tuberculosis Control 2011 report. Available at: http://whqlibdoc.who.int/publications/2011/9789241564380_eng.pdf.Accessed 10 July 2012.

12. Gutierrez EB, Gomes V, Picone CM, Suga H, Atomiya AN. Active tuberculosis and Mycobacterium tuberculosis latent infection in patients with HIV/AIDS. *HIV Med* 2009;10(9): 564-72.
13. World Health Organization(WHO). Global Tuberculosis Report 2012. Available at: http://www.who.int/tb/publications/global_report/en/.Accessed 7 October 2011.
14. Jassal MS, Bishai WR. Epidemiology and challenges to the elimination of global tuberculosis. *Clin. Infect. Dis* 2010;50:156-164.
15. World Health Organization(WHO). Definitions and reporting framework for tuberculosis 2013revision.Availableat:http://apps.who.int/iris/bitstream/10665/79199/1/9789241505345_eg.pdf. Accessed 10 March 2013
16. Ethiopian Ministry of Health (MOH)**.** Guidelines for clinical and programmatic management of TB leprosy and TB/HIV in Ethiopia. Fifth edition, 2012Addis Ababa.
17. Jimma zone health bureau. Annual TB and leprosy prevention and control 2009/10 report.
18. Donald PR, van Helden PD. The global burden of tuberculosis-combating drug resistance in difficult times. *N Engl J Med* 2009;340:669-76.
19. Nachega JB, Chaisson RE. Tuberculosis drug resistance: A global threat. *Clin. Infect Dis* 2003; 36(1):24-30.
20. Johnson R, Streicher EM, Louw GE, Warren RM, van Helden PD, Victor TC. Drug resistance in *Mycobacterium tuberculosis*. *Curr. Iss. Mol. Biol* 2006;8(2): 97-117.
21. Zhang Y, Yew WW. Mechanisms of drug resistance in *Mycobacterium tuberculosis*. *Int J Tuberc Lung Dis* 2009; 13(11):1320-30.
22. Hanif M, Malik S, Dhingra VK. Acquired drug resistance pattern in tuberculosis cases at the state tuberculosis centre, Delhi, India. *Int J Tuberc Lung Dis* **1**3(1)2009;13(1):74-8.
23. Mistrya N, Tolania M, Osrin D. Drug-resistant tuberculosis in Mumbai, India: An agenda for operations research. Oper. Res. Heal. Care 2012;1: 45-53.
24. Gillespie SH. Evolution of Drug resistance in *Mycobacterium tuberculosis*: Clinical and molecular perspective. *Antimicrob. Agents Chemother*. 2002; 46:267.
25. Almeida Da Silva PE, Palomino JC. Molecular basis and mechanisms of drug resistance in *Mycobacterium tuberculosis*: classical and new drugs. *J Antimicrob Chemother*. 2011;66:1417-30.

26. Gandhi NR, Nunn P, Dheda K, *et al*. Multidrug-resistant and extensively drug-resistant tuberculosis: a threat to global control of tuberculosis. *Lancet* 2010;375: 183043.
27. Somoskovi A, Parsons LM, Salfinger M. The molecular basis of resistance to isoniazid, rifampin, and pyrazinamide in *Mycobacterium tuberculosis. Respir Res* 2001;2:164–168
28. Miotto P**,** Saleri N**,** Dembelé M, *et al*. Molecular detection of rifampin and isoniazid resistance to guide chronic TB patient management in Burkina Faso. *BMC Infect Dis* 2009;9:142.
29. Palomino JC. Molecular detection, identification and drug resistance detection in *Mycobacterium tuberculosis*. *FEMS Immunol Med Microbiol.* 2009;1: 1-9.
30. . Kolyva AS, Karakousis PC. Old and New TB Drugs: Mechanisms of action and resistance, understanding tuberculosis: New approaches to fighting against drug resistance. InTech2012;6:948-6.
31. World Health Organization (WHO)–International Union against Tuberculosis and Lung Disease. Anti-tuberculosis drug resistance in the world: 4th global report 2008 . available at: http://www.who.int/tb/publications/2008/drs_report4_26feb08.pdf.Accessed 8 May 2011
32. World Health Organization (WHO).Policy guidance on drug-susceptibility testing of second-line-anti-tuberculosis-drugs.-Geneva-2008. Available at:http://www.who.int/tb/publications/2008/whohtmtb_2008_392/en/index.html.Accessed 9 June 2011.
33. World Health Organization(WHO). Multidrug-resistant tuberculosis 2013 Update. Available at: http://www.who.int/tb/challenges/mdr/MDR_TB_FactSheet.pdf. Accessed 29 February 2012.
34. Yoshiyama T, Shrestha B, Maharjan B. Risk of relapse and failure after retreatment with the category II regimen in Nepal. *Int J Tuberc Lung Dis* 2010;14(11):1428-1423.
35. Quy HTW, Lan NTN, Borgdorff MW, *et al*. Drug resistance among failure and relapse cases of tuberculosis is the standard re-treatment regimen adequate? *Int J Tuberc Lung Dis* 2003;7:631-6.
36. Middelkoop K, Bekker LG, Shashkina E, Kreiswirth B, Wood R. Retreatment tuberculosis in a South African community the role of re-infection, HIV and antiretroviral treatment. *Int J Tuberc Lung Dis* 2012;16:1510-6.

37. Jones-Lopez EC, Ayakaka I, Levin J, *et al.* Effectiveness of the standard WHO recommended retreatment regimen (Category II) for tuberculosis in Kampala, Uganda: A prospective cohort study. *PLoS Med* 2011;8:3.
38. Gler MT, Macalintal LE, Raymond L, Guilatco R, Quelapio MI, Tupasi TE. Multidrug-resistant tuberculosis among previously treated patients in the Philippines. *Int J Tuberc Lung Dis* 2011;15::652-6.
39. Becerra MC, Freeman J, Bayona J**,** *et al.* Using treatment failure under effective directly observed short-course chemotherapy programs to identify patients with multidrug-resistant tuberculosis. *Int J Tuberc Lung Dis* 2000;4:108-14.
40. Namukwaya E, Nakwagala FN, Mulekya F, Mayanja-Kizza H, Mugerwa R. Predictors of treatment failure among pulmonary tuberculosis patients in Mulago hospital, Uganda. *Afr. Health Sci* 2011;11:105 - 11.
41. World Health Organization (WHO). Global tuberculosis Control 2009 report. Available at: http://www.who.int/tb/publications/global_report/2009/key_points/en/.Accessed-25 September 2011.
42. Abate G, Mio¨rner H, Ahmed O, *et al*. Drug resistance in *Mycobacterium tuberculosis* strains isolated from re-treatment cases of pulmonary tuberculosis in Ethiopia: susceptibility to first-line and alternative drugs.Int J Tuberc Lung Dis 1998; 2:580–4.
43. Lemma E, Feleke B, Kebede A, *et al*. Ethiopian. National TB drug resistance surveillance - Ethiopia. progress report. The 8th National TB Research Conference, March 21-24, 2013, Addis-Ababa.-Available-at: http://www.ehnri.gov.et/TRAC%20CON/Day%20Three/2nd%20round%20national%20anti-TB%20progress%20result%20presentation%20TRAC%202013%20(2).pdf. Accessed 9 May 2013.
44. World Health Organization (WHO).Tuberculosis global facts. Available at: www.who.int/tb 2012.accessed 25 April 2012.
45. Abate D, Taye B, Abseno M, Biadgilign S. Epidemiology of anti-tuberculosis drug resistance patterns and trends in tuberculosis referral hospital in Addis Ababa, Ethiopia. *BMC Res Notes* 2012;5:462.
46. Abebe G, Abdissa K, Abdissa A, *et al*. Relatively low primary drug resistant tuberculosis in south western Ethiopia. *BMC Res Notes* 2012;5:225.

47. Ganguly NK, Medappa N, Srivastava VK, *et al*. What is new in the diagnosis of tuberculosis ?Part ii : Techniques for drug susceptibility. *ISSN*2002;32:9.
48. Dongsi Lu, Heeren B, Dunne WM. Comparison of the Automated Mycobacteria Growth Indicator Tube System (BACTEC 960/MGIT) With Löwenstein-Jensen medium for recovery of mycobacteria from clinical specimens. *Am J Clin Pathol* 2002;118:542-5.
49. American Thoracic Society. Diagnostic standards and classification of tuberculosis in adults and children. *Am J Respir Crit Care* Med 2000;161:1376-95.
50. Warns M, Carrese ED, Beaty PS. Comparison of the BACTECTM MGIT 320 to the BACTEC MGIT 960 for the growth detection and susceptibility testing of *Mycobacterium tuberculosis*. As presented at the 110th General Meeting of the *ASM,* San Diego, CA, 2010.Avialable-at: http://www.bd.com/ds/technicalCenter/whitepapers/lr223279.pdf.Accessed on 20 march 2013.
51. Said HM, Kock MM, Ismail NA, *et al.* Comparison between the BACTEC MGIT 960 system and the agar proportion method form susceptibility testing of multidrug resistant tuberculosis strains in a high burden setting of South Africa. BMC Infec. Dis. 2012;12:369.
52. World Health Organization (WHO). Molecular line probe assays for rapid screening of patients at risk of multidrug-resistant tuberculosis (MDR-TB). Policy statement, 2008.Avilable at: http://www.who.int/tb/features_archive/policy_statement.pdf. Accessed 12 March 2012.
53. Nyendak MR, Lewinsohn DA, Lewinsohn DM. New diagnostic methods for tuberculosis. *Curr Opin Infect Dis* 2009,;22:174-82.
54. Hazbon MH. Recent advances in molecular methods for early diagnosis of tuberculosis and drug resistant tuberculosis. *biomedica*2004;24:149-62.
55. Xpert® MTB/RIF. Two-hour detection of MTB and resistance to rifampicin. 2010. availableat:http://www.cepheid.com/media/files/eu/brochures/Xpert_MTBRIF%20Brochure%20EU%200089-02%20LOR.pdf. Accessed 14 March 2012.
56. Blomberg B, Spinaci S, Fourie B, Laing R. The rationale for recommending fixed-dose combination tablets for treatment of tuberculosis. *Bull World Health Organ* 2001;79(1):61-8.

57. World Health Organization(WHO). Treatment of tuberculosis Guidelines. Fourth edition 2010.-Available-at: http://whqlibdoc.who.int/publications/2010/9789241547833_eng.pdf.Accessed 3 July 2011.
58. Mukherjee A, Sarkar A, Saha I, Biswas B, Bhattacharyya PS. Outcomes of different subgroups of smear positive retreatment patients under RNTCP in rural West Bengal, India. *Rural Remote Health*. 2009;9(1):926.
59. Ethiopian Ministry of health(MOH). Guideline for program and clinical management of drug resistant tuberculosis. First edition, 2009.
60. World Health Orhanization(WHO). Treatment of tuberculosis Guidelines. Fourth edition 2009.-Available at:http://whqlibdoc.who.int/publications/2009/9789241547833_eng.pdf.accessed 12 June 2012
61. Falzon D, Jaramillo E, Schu¨nemann HJ, *et al.* WHO guidelines for the programmatic management of drug-resistant tuberculosis 2011 update. *Eur Respir J* 2011;38:516-28
62. Drobniewski F, Ru¨sch-Gerdes S, Hoffner S. Antimicrobial susceptibility testing of *Mycobacterium tuberculosis* (EUCAST document E.DEF 8.1) report of the subcommittee on antimicrobials susceptibility testing of *Mycobacterium tuberculosis* of the European committee for antimicrobial susceptibility testing (EUCAST) of the European society of clinical microbiology and infectious diseases (ESCMID). *Clin Microbiol Infect* 2007; 13: 1144–1156.
63. Zai S, Haroon T, Mehmood KT. Socioeconomic Factors Contributing to Multidrug-Resistant Tuberculosis (MDR-TB). *J Biomed Sci and Res*2010;2:2(4)2:79-83.
64. Schreiber YS, Herrera AF, Wilson D, *et al.* Tuberculosis retreatment category predicts resistance in hospitalized retreatment patients in a high HIV prevalence area. *int J Tuberc Lung Dis* 2009;13:1274-80.
65. Wright A, Zignol M, Van Deun A, *et al.* Epidemiology of anti-tuberculosis drug resistance 2002–07: an updated analysis of the global project on anti-tuberculosis drug resistance surveillance. *Lancet* 2009; 373:1861-73.
66. Pooran A, Pieterson E, Davids M, Theron G, Dheda K. What is the cost of diagnosis and management of drug Resistant tuberculosis in South Africa? *PLoS ONE*2013; 8(1).

67. GBC-Health.-Drug-resistant-TB:-why-it-matters-2011.availble-at: http://www.gbchealth.org/system/documents/category_1/7/GBCHealth%20Issue%20Brief_Drug-Resistant%20TB.pdf.Accessed 25 March 2012.
68. Abubakar I , Zignol M, Falzon D, *et al*. Drug-resistant tuberculosis: time for visionary political leadership. *Lancet Infect Dis* 2013;13(6): 529 - 539
69. Yimer AS, Agonafir M, Derese Y, Sani Y, Bjune GA, Holm-Hansen C. Primary drug resistance to anti-tuberculosis drugs in major towns of Amhara region, Ethiopia. *APMIS*2012;120(6): 503–509.
70. Sachdeva KS, Satyanarayana S, Dewan PK, *et al.* Source of Previous Treatment for Re-Treatment TB Cases Registered under the National TB Control Programme India, 2010. *PLoS ONE*2010; 6:(7).
71. Mak A, Thomas A, del Granado M, Zaleskis R, Mouzafarova N, Menzies D. Influence of Multidrug Resistance on Tuberculosis Treatment Outcomes with Standardized Regimens. *Am J Respir Crit Care Med* 2008;178: 306-12.
72. Temple B, Ayakaka I, Ogwang S *et al*. Rate and Amplification of Drug Resistance among Previously-Treated Patients with Tuberculosis Kampala,Uganda. *Clin. Infect. Dis*. 2008; 47 (9): 1126-1134.
73. Schoch OD, Rieder HL. Characteristics of sputum smear-positive tuberculosis patients with and without HIV infection in a hospital in Zimbabwe. *Eur Respir J*, 1996;9: 284-7
74. Sharma SK, Kaushik G, Jha B, *et al.* Prevalence of multidrug-resistant tuberculosis among newly diagnosed cases of sputum-positive pulmonary tuberculosis. *Indian J Med Res* 2011;133:308-11.
75. Jenkins HE, Zignol M, Cohen T. Quantifying the burden and trends of isoniazid resistant tuberculosis, 1994–2009. *PLoS ONE*2011;6:7.
76. Ndung'u PW, Kariuki S, Ng'ang'a Z, Revathi G. Resistance patterns of *Mycobacterium tuberculosi*s isolates from pulmonary tuberculosis patients in Nairobi. *J. Infect. Dev. Ctries* 2012;*6* .33-9.
77. Hoek KG, Schaaf HS, van Pittius NC, van Helden PD, Warren RM. Resistance to pyrazinamide and ethambutol compromises MDR/XDR-TB treatment. *SAMJ*2009;99.

78. Merza MA, Farnia P, Tabarsi P, Khazampour M, Masjedi MR, Velayati AK. Anti-tuberculosis drug resistance and associated risk factors in a tertiary level TB centre in Iran: A retrospective analysis. *J Infect Dev Ctries* 2011;5:511-9.
79. Liang L, Qunhong Wu, Lijun Gao, *et al*. Factors contributing to the high prevalence of multidrug-resistant tuberculosis: a study from China. *Thorax* 2012.Availble at: thorax.bmj.com . accessed on 4 may 2013.
80. Migliori GB, Dheda K, Centis R, *et al*. Review of multidrug-resistant and extensively drug-resistant TB: global perspectives with a focus on sub-Saharan Africa. *Trop Med Int Health* 2010;15(9):1052-66.
81. Sharma SK, Kumar S, Saha PK, *et al*. Prevalence of multidrug-resistant tuberculosis among Category II pulmonary tuberculosis patients. *Indian J Med Res* 2011;133:312-5.
82. World health organization(WHO). Guidelines for surveillance of drug resistance in tuberculosis . 2009 Fourth Edition

CHAPTER SIX

ANNEXES

6.1 ANNEX I

6.1.1 questionnaire

Questionnaires to be used to gather clinical and laboratory information to determine the in vitro drug resistance patterns of *M. tuberculosis* among retreatment TB infected patients

Diagnostic Centre: _____ TB lab Code_____

1. Identification of the Patient
   A. Name: _____ Date registered: Day---- Mo------Yr-
   B. Sex: 1. Male
      2. Female
2. Age: _____ years ,Address_____ Card No_____
3. Date of sputum collection: _____month _____year

Sputum smears examination, HIV status ART status

	Observed Response	Coded response 1=yes 0=no
4. Result of smear examination 4.1. 1st specimen	1. positive for AFB 2. negative for AFB	
4.2. 2nd specimen	1. positive AFB 2. negative AFB	
4.3. 3rd specimen	1. positive AFB 2. negative AFB	
5 .HIV-sero status	1. Positive for RVI 2. Negative for RVI	
5.1 If positive for RVI ,	1. Currently on ART	

ART status of the patient	2. Not on ART	

6. Previous history of tuberculosis disease

	Response from interview	Coded response (1=Yes 0= No)
6.1.Previous history of tuberculosis	1.yes 2. no	
6.2. Duration of illness	1. One month 2.Two months 3. ≥ three months	
6.3. Other symptoms of lung disease prior to this episode	1. Yes 2. No	
6.4 cough	1. Yes 2.No	
6.5. chest pain,	1.yes 2.no	
6.6 haemoptysis,	1.yes 2.no	
6.7 Night sweats	1.yes 2.no	
6.8 Fatigue/tiredness	1.yes 2.no	
6.9 Shortness of breath	1.yes 2.no	
6.10 weight loss	1.yes 2.no	
6.11 . Fever	1.yes 2.no	

7. Clinical history

	Response from interview	Coded response (1=Yes 0=No)
7.1 .X-ray examinations prior to this episode	1. Yes 2. No	
7.2. Sputum examinations prior to this episode	1. Yes 2. No	
7.3. Anti- tuberculosis drugs for more than one month	1. Yes 2. No	
7.4. Injections for more than one month	1. Yes 2. No	

8. Previous history of treatment

A. Previous treatment center _____

B. Treatment date _____

C. Number of times the patient treated _____

D. Retreatment sub –categories 1. Relapse 2. Default 3. Treatment failure 4. Chronic case

9. Predisposing factors for tuberculosis

	Response	Coded response
11.1. Diabetes	1. yes 2. No	
11.2. Cigarette smoking	1. yes 2. No	
11.3. Alcohol consumption	1. yes 2. No	
11.4. History of being prisoned	1. yes 2. No	
11.5.Chronically coughing individual in the family	1. yes 2. No	

10. Culture result

A. Positive: 1. Yes 2. No

B. Contaminated: 1. Yes 2. No

11. Identification

A. MTBC 1.yes 2.No

B.NMT 1.yes 2.No

12. Results of antibiotic sensitivity testing

(Patient ID _____)

	Previously treated cases	
	Sensitive	**Resistant**
Sensitive to all four drugs		
Isoniazid (H)		
Rifampicin (R)		
Ethambutol (E)		
Streptomycin (S)		
MONORESISTANCE		
Isoniazid (H)		
Rifampicin (R)		
Ethambutol (E)		
Streptomycin (S)		
MULTIDRUG RESISTANCE		
H + R		
H + R + E		
H + R + S		
H + R + E + S		
OTHER PATTERNS		
H + E		
H + S		
H + E + S		
R + E		

6.2 Annex II

6.2.1 Information sheet read to the respondents

My name is **Kedir Abdella** MSC student of Jimma University, college of public health and medical science, department of medical laboratory science and pathology

Institute: Jimma University

Introduction: This information sheet is prepared by the groups investigators that are formed to conduct study with aim looking the drug resistance pattern of M. *tuberculosis* against first line anti-tuberculosis drugs among retreatment tuberculosis cases at Jimma University specialized hospital . The group consists of two advisors from Jimma University, college of public health and medical sciences, department of medical laboratory science and pathology and principal investigator final year postgraduate student in Clinical microbiology .

Purpose

The purpose of this study is to find out drug resistance patterns of *M.tuberculosis* in smear positive retreatment cases. In this study we will be collect information regarding socio demographic characteristics, clinical finding and common possible predisposing factors of TB. This study will use various laboratory methods to looks into the extent the drug resistance (monodrug resistance ,multidrug resistance and poly drug resistance) pattern of M. *tuberculosis* for first line drugs in Jimma zone .furthermore , the study will look in to the drug resistance patterns of isolates among sub –categories of retreatment case (relapse, treatment failure ,defaulter and chronic cases)

Procedure

The laboratory analysis will be conducted in Jimma university specialized hospital TB laboratory. The study will be conducted through analysis of sputum specimens. Thus would like to kindly request you to participate in this study that intended to looks into drug resistance patterns of M.tuberculosis in retreatment \ cases .

A total of 84 people currently smear positive and confirmed as previously treated cases for at least one month will be asked to take part in this research project. The study will take 3 months. If you take part in this research, the time it takes to be giving us information is once a time

during your stay in hospital. Therefore, there will not be fear of spending extra time in your part due to the study.

If you are willing to participate in the study, you will be asked to sign a consent form. You are only required to sign after you are given full explanation and received answer to your queries. You will be asked to give consent for taking a small amount of sputum specimen by standard procedure to collect intended sputum. Safely collected specimen will be transported to Jimma university specialized hospital TB laboratory which is in study setting for further examination. We would like to explain that the procedures generally accepted and used to conduct drug susceptibility testing of *M, tuberculosis* from sputum specimen.

The information you provide will be used to improve TB control and to design appropriate public health interventions for future. Your answers will not be released to anyone and will remain anonymous. Your name will not be written on the questionnaire or be kept in any other records. Your participation is voluntary and you may choose to stop the interview at any time. Your participation or not participation will not have any influence for your service that you want to use .In addition in your participation in the study may or not have any invasive procedure, each questionnaire will be only take 10-15 minutes. Drug study time your specimen result will be reported to your corresponding physician for further contribution in your treatment and clinical management. Study results of with drug resistant strains particularly MDR-TB will be announced to hospital administration and hospital TB clinic office and will be treated by announce to concerned body.

Risk and discomfort

Benefits

If you participate in this research, you will get the following benefits. There will be no payment for the laboratory expenses.

If you are found to be positive for MDRTB, we will timely communicate with TB clinic for the initiation of treatments and any supportive management for patients with MDR TB

.**Incentive**

You will not be given any incentive except for the benefits mentioned above.

Right to refuse or withdraw

Your participation in this research is wholly dependent on your willingness to do so. It will in no way affect your right to acquire health care and treatment if you do not wish to participate in the

project. You will still have all the benefits that you would have and will not affect your treatment at this centre in any way. Your right to discontinue participating in this research and withdraw your consent will be respected.

Who to contact

This proposal has been reviewed and approved by the Jimma university college of public health and medical science Research review Ethical Committee. The task of these committees is to make sure that research participants are protected from harm. If you will be have any questions you can ask now or in the future. If you wish to ask questions later, you may contact any of the following

Kedir Abdella principal investigator telephone +251913 158356. Email: kedir.abdella@ju.edu.et

Mr. Gemeda Abebe research adviser telephone: +251 911991285.

Email: gemeda.abebe@ju.edu.et

Mr. Ketema Abdissa research adviser telephone +251912035503.

Email:ketema.abdissa@ju.edu.et

For the successes of our study, we will be asking to give correct answer for respective questions.

Thank you for your assistance. Continue answering those questions

6.3 Annex- III

6.3.1 Patient consent form

Name of the Institute_____

Card No. _____

I have been informed fully in the language I understand about a research project that aims to determining drug resistance patterns of MTB among retreatment tuberculosis case take part in study in Jimma university specialized hospital . I have been asked to participate in the study voluntarily. If I take part in the research, I will give 2-5ml sputum specimen. It is explained to me that this procedure is the generally accepted method for accurate testing of drug resistance in of MTB.

The procedure is done free of charge and the results will be made available for my physician and I will receive treatment and other clinical management for free. Any complication arising from the procedure will be taken care of by the projects' physician. It is also explained to me that if I prefer not to participate in the study, I will still receive all the care required for my condition

without any prejudice to me. I can also withdraw my consent at any time I want. My personal information, clinical finding and laboratory results will not be given to anyone else without my permission. If I happen to be infected with drug resistant TB particularly MDR TB, I will be provided with the best national standard treatment and clinical managements for drug resistant TB (MDRTB). I am aware that this project will not provide any extra intensive for my participation in the study.

I have been asked to give my consent for the leftover materials taken from my body for research on drug resistance of MTB among retreatment cases and to be stored at JUSHTBL to answer relevant questions that might arise at a later date. All my clinical information will be kept in strict confidence.

I agree to participate in the study after receiving clarification for my questions and sufficient time to think about the request. By participating in this project, I have contributed to the better diagnosis of drug resistance testing and management of MDR-TB and lent a hand in the struggle that is ongoing to eradicate TB in our country.

Name of the patient _____

Signature Witness signature_____date_____

Uunkaa Informeshiinii Hirmaatota qorannootif dubifamu

Ani maqaan kiyya **Kadir Abdallaa** jedhama**.** Yunivarsitii Jimmaa, Dipaartimantii saaynsii laaboraatorii fayyaa fi paatologiitti barataa digirii lammafati

Maqaan dhabbataa: Yunivarsitii Jimmaa

Sensa : uunkan Informeshiinii kun garee hayyu duree qo,anna kana Kedir Abdella fi gorsitotaa lama obbo Gamadaa Abbabaa fi Oboo Katamaa Abdisaatiin kan qophaheedha .

kayyoo: kayyoon qo'anna kanaa halaa baakteriyaan dhukkuba daranyoo sombaa fidu qoricha farra dhukkuba sombaatiin walbaruun isaa dhukkusatotta dhibee somban qabamaniif armaan dura qiricha farra dhukkuba sombaa fudhatan jiddutti maal akka fakkatu ilaaludha .Qo'anna kana keessatti ragaleen hawasumma fi medicalaa dhukkuba kanaaf sababa tahu danda'an ni funaanamu. . Qo'annicha kana keessati akkeen namoota kana irra akka fudhamuun qorannon laaboraatorii adda addaa ni tasifama.

Adeemsa

Qorannon laaboraatorii adda addaa qo'anna kanaa yunivarsitti jimmaa kuta laaboraatorii maaykoobaakteriyolojitti kan ademsifamu taha. Qorannon kan tasifamu akkee isin irra fudhatamu irratti waan tahef gama kessaniin qo'anna kana kessatii fedhiin akka nu hirmaatan kabajan isin gafanna .

Qo'annich kan rawatamuu walii gala namoota 78 irratti yoo tahuu hanga ji'oota shanii ni fudhata jedhameetu yadama. Isin garuu yoo qo'anna kanatti hiraattan yeroon itti ragaa barbaachisuu fi akkee nuuf kennitan yeroo tokko yalaaf gara hospitaala jimma dhuftan qofadha . kanafuu sodaan yeroo keessan baayee balleessa jedhu hin jiratuu.

Yoo qo'annaa kanatti hirmaatuuf fedhii kessan nuuf kennitan mallattoo fedhii keessan kana mirkaneessu akka nuuf mallatesitan kabajaan isin gaffachuu barbanna. Mallatto kana kan mallateesitan erga ibsi gahan isinii kennamee fi deebiin gaafii keessanii isiif kennameedha. Mallatto fedhii kessanii kan nuuf kennitan ragaalee isin irra sassabamuu fi akkee qoranno laaboraatoriitiif nuuf kennitanif taha.Ragallen isiin nuuf kennitan fooyya'insa to'anna dhukkuba daranyoo sombaatiif eddo gudda qaba.Deebiin isin gaafii kennaaf deebistan kamiyyuu qamaa biraatiif dabarsinee hin kenninu .

Hirmaanaan kessan kamiyuu fedhii irratti kan hundaahee fi yeroo barbaddanitti gafii fi deebii kana addaan kutuu kan dandeettan tahuu isif ibsuu barbanna.Gama biraatiin qo'nna kanatti

hirmaachuun kessaan midhaa tokkolle kan hin qabne yoo tahu gafii deebiin kun daqiiqaa 10-15 qofa kan fudhatuudha .Firiin Qoranno laaboraatorii akkee keessanii akka yaalaaf tolu uf doktora kessanitti gabasa isin gona. Yoo qorannon firii laaboraatorii kun baakteeriyaa qoricha farra dhukkuba sombaan hedduun walbare(MDR-TB) muldhise kana hattamman gama qama dhimmi ilaalutti kan gabafnuu tahuu ibsina .

Balaa fi midhaa Faydaa

Yoo qo'anna kanatti hirmattan faydalee arman gadii kana argattu

Qoranno laaboraatoriitiif kaffaltii hin gafatamtan .

yoo baacteriyaa qoricha farra dhukkuba sombaa baayeen walbareen qabamtan yaalii akka tolutti hatattaman gara kilinikii yala dhukkubsatota sombaatti gabasa gona .

.**Kaffaltii**

faydalee arman olitti eeramanin alatti kaffaltiin adda hin jiru.

Mirga didu ykn addan kutuu

Qo'anna kanatti hirmaatuun keessan gutuun gututti fedhii keessan irratti kan hundahedha. Diduufis tahee addan kutuuf mirga gutuu qabdu . Hirmannan kessan fayidaa yala isin argatuu qabdaniin walitti hidhata hin qabu . Hirmatanus dhiftanuus mirga yalamuu qabdan isin hin dhabsisuu .

Odeefannoo dabalataa

Ademsi qo'anna kana garee boordii riviwuutiin yunivarsitii jimmaa kollajii fayya hawasaa fi saaynsii fayyaatin kan mirkana'eedha . Gaffii yoo qabattan gaffachuu dandessu. Gaffiilee dabalattaatif namotaa armaan gaditti eeraman kana haasofsisuu ni dandessu.

1 Obbo Kedir Abdella nama qo'anna kana ademsisu lakk. bilbila +251913 158356. Email: kedir.abdella@ju.edu.et

2.Obbo Gemeda Abebe gorsaa qo'annicha lakk. bilbila: +251 911991285.

Email: gemeda.abebe@ju.edu.et

3 .Obbo. Ketema Abdissa gorsaa qo'annich lakk. bilbila +251912035503. Email:ketema.abdissa@ju.edu.et

milka'anna kanatiif gaffiilee armaan gadiitiif deebii sirrii tahe akka nuf kennitan kabajaan isin gaffana .

Galatomaa!!!. Gaffiin ittii fufa

uunkaa Hirmatotin fedhidhan hirmatuu isaanii ittin mirkaneesan

Maqaa dhabbata fayyaa _____

lakk. kaardii _____

kayyoo qo'anna yunivarsitii jimmatti haala qoricha farra-dhukkuba sombaatiin walbaruu baakteeriyaa dhukkuba somba fiduu kana qonqaa ani dhagahuun akka gariitti naf ibsamee jira .Haaluma kanaan fedhii kootin qo'anna kanatti akka hirmadhuuf gafatamee jira . Yoo qo'anna kanatti hirmadhe haala ajaja oogeessa fayyaatin akkee 2ml -5ml tti akka kennu naaf ibsamee jira.qorannan laaboraatorii akkee kiyya irratti naaf tasifamu kaffaltii irra bilisa akka tahe naaf himamee jira .kanamalees firiin qoranno laaboraatorii yeroodhan gara doktora kiyyaan naaf ergamuun yaalii barbachisa tahe akka argadhuu naaf ibsamee jira .

Gama biraatiin yoo fedhii qo'anna kanatti hirmaachuu hin qabane ammas tanaan tajaajila argachuu narra malu akka argadhuuf hirmaachuuf hirmaachuu dhisuun kiyya tajaajila argachuu qabuun akka walitti hidhata hin qabne hubadhee jira .yeroo barbadetti addan kutuu akka danda'u naaf ibsame jira . Ragaan enyumma kiyyas tahe kan qoranno laaboraatorii eeyyama kiyyan alatti dabarsanii eennumaafuu akka hin latamnne natti himanii jiru .yoo akka tasaa baakteeriyaan qiricha farra dhukkuba somdaa heddun walbare na keessatti argame yaalii sadarkaa biyyooleesaa eegatee akka argadhu naaf ibsamee jira . kaffaltiin addaa hirmanna qo'anna kanaa irraa argadhu akka hin jirre naaf ibsamee jira .

Haaluma kanaan fedhii kiyyan qo'anna kanatti hiramachuuf murteesse jira .kana gochuu kiyyan tarkaanfii dhukkuba sombaa to'achuuf tasifamu kessatti qooda fudhachuun gahee narraa eegamu bahuu barbaada . fedhiidhaan hiraamachu kiyya mallaatto itti anu kanaan mirkaneessu barbada

Maqaa hiramataa _____

mallattoo_____Guyyaa_____

የ መ ረ ጃ ቅ ፅ

ስ ሜ ከ ድ ር አ ብ ደ ላ ይ ባ ላ ል በ ጅ ማ ዩ ኒ ቨ ር ሲ ቲ የ ህ ብ ረ ተ ሰ ብ ጤ ና ህ ክ ም ሳ ይ ን ስ ኮ ሌ ጅ የ ድ ህ ረ -ም ረ ቃ ተ ሜ ነ ኝ ፡ ፡

ድ ር ጅ ቱ ፡ ጂ ማ ዩ ኒ ቨ ር ሲ ቲ

መ ግ ቢ ያ ፡ ይ ህ የ መ ረ ጃ ቅ ፅ የ ተ ዘ ጋ ጀ ው በ 3 ተ መ ራ መ ሪ ዎ ች ሲ ሆ ን አ ላ ማ ው ም መ ድ ህ ኒ ት የ ተ ላ መ ደ የ ሳ ን ባ ነ ቀ ር ሳ በ ሽ ታ የ ሚ ያ መ ጣ ባ ክ ቴ ሪ ያ ስ ር ጭ ት ና ተ ያ ያ ዝ ስ ት ያ ላ ቸ ው ን ም ክ ን ያ ቶ ች ን ማ ጥ ና ት ይ ሆ ና ል ፡ ፡ በ ዚ ህ ጥ ና ት ወ ስ ጥ ማ ህ በ ር ሰ ባ ዊ ጥ ያ ቄ ዎ ች ና ጤ ን በ ተ መ ለ ከ ተ መ ረ ጃ ይ ሰ በ ስ ባ ል ፡ ፡ በ ተ ጨ ማ ሪ ም ለ ላ ቦ ራ ቶ ሪ ም ር መ ራ የ ሚ ሆ ን የ አ ከ ታ ና መ ኛ እ ን ወ ስ ዳ ለ ን ፡ ፡

አ ካ ሄ ድ ፡ የ ላ ቦ ራ ቶ ሪ ም ር መ ራ የ ሚ ከ ሄ ደ ው በ ጅ ማ ዩ ኒ ቨ ር ሲ ቲ የ ሳ ን ባ ነ ቀ ር ሳ ም ር መ ራ ላ ቦ ራ ቶ ሪ ሲ ሆ ን በ ዚ ህ ጥ ና ት እ ን ዲ ሳ ተ ፉ ስ ን ል በ ት ህ ት ና እ ን ጠ ይ ቃ ለ ን ፡ ፡ በ ጠ ቅ ላ ላ ው 84 በ አ ከ ታ ቸ ው ወ ስ ጥ የ ሳ ን ባ ነ ቀ ር ሳ የ ሚ መ ጣ ባ ክ ቴ ሪ ያ የ ተ ገ ኘ ባ ቸ ው ህ መ ማ ን በ ዚ ህ ጥ ና ት ተ ሳ ታ ፊ ይ ሆ ና ሉ ፡ ፡ በ ዚ ህ ጥ ና ት ተ ሳ ታ ፊ ከ ሆ ኑ መ ረ ጃ እ ና የ አ ከ ታ ና መ ኛ የ ሚ ሰ ጠ ው አ ን ድ ጊ ዜ ብ ቻ ስ ለ ሆ ነ ይ ህ ን ጥ ና ት ተ ጨ ማ ሪ ጊ ዜ ያ ች ሁ ን እ ን ድ ታ ጠ ፉ አ ያ ደ ር ግ ም ፡

በ ዚ ህ ጥ ት ለ መ ሳ ተ ፍ ፍ ቃ ደ ኛ ከ ሆ ኑ የ ስ ም ማ ት ወ ረ ቀ ት እ ን ዲ ፈ ር ሙ ይ ጠ የ ቃ ሉ ፡ ፡ ይ ህ ን የ ሞ ሉ ደ ር ጉ ት ስ ለ ጥ ና ቱ በ ቂ የ ሆ ነ መ ረ ጃ ከ ተ ሰ ጠ ዎ ት በ ኋ ላ ነ ው ፡ ፡ የ ና መ ኛ አ ወ ሳ ሰ ዱ ሳ ይ ን ሳ ዊ ቅ ደ ም ተ ከ ተ ሎ ች ን የ ተ ከ ተ ለ ነ ው ፡ ፡ እ ር ሶ ዎ የ ሚ ሰ ጠ ው መ ረ ጃ የ ሳ ን ባ ን ነ ቀ ር ሳ በ ሽ ታ ለ መ ቆ ጣ ጠ ር በ ሚ ደ ረ ገ ው ር ብ ር ብ ወ ስ ጥ ጉ ል ህ አ ስ ተ ዋ ፅ ኦ አ ለ ው ፡ ፡ ስ ለ ሆ ነ ም በ ተ ቻ ለ ማ ክ ን ት ክ ክ ለ ኛ ው ን መ ረ ጃ እ ን ዲ ሰ ጠ ኑ በ ት ህ ት ና እ ን ጠ ይ ቃ ለ ን ፡ ፡

በ ጥ ና ቱ የ ሚ ኝ ው ጥ ቅ ም ፡ በ ዚ ህ ጥ ና ት የ ሚ ሳ ተ ፉ ከ ሆ ነ የ ሚ ከ ተ ሉ ት ን ጥ ቅ ሞ ች ያ ገ ኛ ሉ ፡

1. ነ ፃ የ ላ ራ ቶ ሪ ም ር መ ራ
2. ነ ፃ የ ህ ክ ም አ ገ ል ግ ሎ ት

ክ ፍ ያ ፡ በ ዚ ህ ጥ ና ት በ መ ሳ ተ ፍ ዎ የ ሚ ከ ፍ ሉ ት ም ን ም አ ይ ነ ት ነ ገ ር የ ለ ም

የ ተ ሳ ታ ፊ ው መ ቡ ት ፡ ወ ድ የ ጥ ና ቱ ተ ሳ ታ ፊ ዎ ች ይ ህ ጥ ና ት የ ሚ ከ ሄ ደ ው በ ና ን ተ መ ል ካ ም ፍ ቃ ድ ብ ቻ ነ ው ፡ ፡ መ ሳ ተ ፍ ካ ል ፈ ለ ጉ በ ሆ ስ ፒ ታ ሉ የ ሚ ገ ኙ ት ማ ን ኛ ው ም የ ህ ክ ም አ ገ ል ግ ሎ ት ላ ይ ተ ፅ ዕ ኖ የ ለ ው ም በ ተ ጨ ማ ሪ ም በ ፈ ለ ጉ ት ሰ አ ት ጥ ና ቱ ን የ ሚ ቋ ረ ጥ መ ብ ት አ ለ ዎ ት ፡ ፡

ለተጨማሪ መረጃ ከዚህ በታች የተጠቀሱትን ሰዎች ማነጋገር ይችላሉ፡፡

1. ከድር አብደላ፡ ዋና ተመራማሪ

   ስ.ቁ፡ 09 13 15 83 56

   Email: kedir.abdella@ju.edu.et

2. ዶ/ር ገመዳ አበባ፡ - አማካሪ

   ስ.ቁ፡ 09 11 99 12 87

   Email: gemeda.abebe@ju.edu.et

3. አቶ ከተማአቡዲሳ፡ አማካሪ

   ስ.ቁ፡ 09 12 03 55 03

   Emai: ketema.abedessa@ju.edu.et

የስምምነት ቅፅ

የመከሉ ስም_____

የካርድ ቁጥር_____

ስለጥናቱ አላማ መሉ ለመሉ ተረድቻለሁ፡፡ በጥናቱ ላይ በፈቃደኝነት እንደሳተፍ ተጠይቄአለሁ፡፡ በዚህ ጥናት የምሳተፍ ከሆነ ከ2-5 ml የሜርስ የአክታ ናሙና እሰጣሁ፡፡ ጥናቱን በተመለከተ ሳይንሳዊ የሆነ የምርመራ ቅደም ተከተሎች እንደሚከተሉ ተረድቻለሁ፡፡

ለላቦራቶሪ ምርመራውም ሆነ ለህክምና ማመጣውምንም አይት ወጪ(ክፍያ) እንደሌለብኝ ተነግሮኛል፡፡ በተጨማሪም በጥናቱ ለመሳተፍ ፍቃደኛ ካልሆን መተውእንደምችል አስረድተውኛል፡፡

ማቋረጥም ከኔ የሚመሰድ መረጃ እና ናሙና ለተፈለገው ጥናት ብቻ እንደሚውል አስረድተውኛል፡፡ መድኃኒትን የተላመደ ሳንባ ነቀርሳ የሚያመጣባክቴሪያ ከተገኘብኝ አስፈላጊውን የህክምና አገልግሎት በነፃ እንደማገኝ ተገልፆልኛል፡፡

አስፈላጊ ማበራሪያና በቂ ገለፃ ከተደረገልኝ በኋላ በዚህ ጥናት ለመሳተፍ ፈቃደኛ ነኝ፡፡

የተሳታፊ ስም_____ፊርማ_____ቀን_____

6.4 ANNEXES IV

6.4.1 *Laboratory procedure*

Specimen collection

- ❖ Give the patient confidence by explaining to him/her the reason for specimen collection
- ❖ collect the sufficient appropriate sputum specimen
- ❖ Check that the container is securely closed and label the container (not the lid) clearly
- ❖ Wash hands with soap and water
- ❖ Demonstrate to the patient how the container should be securely closed

Ziehl Neelsen methods: Procedure

Prepare smear as describes; allow air to dry.

Heat fix smear by passing the slide 2x or 3x over flame

Flood the slide carbol fuchsine

Heat the slides with flame until the vapour appeared and wait the for 5' and wash smear with water

Flood smear with acid alcohol, allow destaining for 2 minutes

Wash smear again with water

Floods slides with ethylene blue and counter stain for 1 to 2 minutes

smear again with water and air dry .do not blot

Examine smears under oil immersion objectives lens of the microscope

Examination of smears

The smear should be searched in an orderly manner by making a series of three parallel or nine sweeps the length of smears or nine parallel sweeps the width of the smears.

Reporting of smear results

Smear result should be quantified to be meaningful.

N^o of AFB seen	report
None……..	No AFB seen

1-2/300 fields……..±

1-9/100 fields……. …….1^+

1-9 /10 field's……2^+

1-9 /fields……..3^+

>9 /fields……..4^+

2). **N-acetyl L-cysteine- Sodium hydro oxide method**

NaOH is toxic, both for contaminants and also for tubercle bacilli; therefore, strict adherence to the indicated timings is required.

Reagents: NALC-NaOH: 1% and Phosphate buffer 0.067M, pH 6.8

Procedure:

Step1-Weight 4g NaOH in 100 ml distill water

Step2- Weight 2.9 g sodium citrate in 100 ml distill water

Step 3- Mix step 1 &2

Step 4- Add 0.5g NALC

Specimen processing

Step 1- transfer the specimen (at least 2 ml, not more than 5 ml) in to a centrifuge

Step 2- add equal volumes of NALC-NaOH solution

Step 3- tighten cap of container and vortex slowly

Step 4-shake intermittently to aid homogenization and decontamination

Step 5-invert each bottle to ensure that NaOH solution contacts all the sides and inner portions of caps

Step 6-keep at 20 ^{o}c -25^{o}c for 15 min for decontamination

Step 7-fill the tube with phosphate buffer up to 50 ml mark on the tube

Step 8- vortex

Step 9- centrifuge at 3000g for 15 min

Step 10-carfully pours off the supernatant in to a discarded can containing 5 % phenol or other germicide

Step 11-inoculate 0.1ml of deposit on to two slopes of LJ medium or 0. 5ml deposit in to MGIT tube labeled with the ID number

Step12-use a pipette inoculate each slope with 3 to 4 drops (100µl) for LJ and 0.5µl for each MGIT tube

Step 13-smear on a slide with the ID number for microscope examination

4. Preparation of egg-based PNB containing LJ media

PNB containing LJ medium favors the growth of NMT while it prevent the growth of MTBC. It should be used in countries or regions where patients may be infected with either organism. And prepared according to standard operating procedure for LJ media preparation .

Ingredients:

A) Mineral salt solution:

-Potassium dihyderogen phosphate anhydrous (KH2PO4) ---2.4g

-Magnesium sulphate (MgSO47H2O) 0.2g

-Magnesium citrate 0.6g

-Asparagines …….3.6g

Glycerol (reagent grade)……...12ml

-Distilled water……..600 ml

NB: Dissolve the ingredients in the distilled water by heating, autoclave at 121^{oc} for 30 minutes to sterilize. Cool to room temperature. This solution keeps indefinitely and may be stored in suitable amounts in the refrigerator.

B) Malachite green solution:

-Malachite green dye……2.0g

-Sterile distilled water …….100ml

-PNB ----------25g

NB: Using aseptic techniques dissolve the dye in sterile distilled water by placing the solution in the incubator for 1-2 hours. This solution will not store indefinitely and may precipitate or change to a less-deeply colored solution. In either case discard and prepare a fresh solution.

c). Homogenized whole eggs

Scrubbing thoroughly with a hand brush in warm water and a plain alkaline soap cleans fresh hens' eggs, not more than seven days old. Let the eggs soak for 30 minutes in the soap solution. Rinse eggs thoroughly in running water and soak them in 70% ethanol for 15 minutes. Before handling the clean dry eggs scrub the hands and wash them. Crack the eggs with a sterile knife into a sterile flask and beat them with a sterile egg whisk or in a sterile blender.

d). Preparation of complete medium

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

Mineral salt solution…….600 ml

Malachite green solution …….20 ml

Homogenized eggs (20-25 eggs, depending on size….1000ml

Finally the complete egg medium is distributed in 6-8ml volumes in sterile 14ml or 28ml McCartney bottles or in 20ml volumes in 20 x 150mm screw-capped test tubes, and the tops are securely fastened.

e). **Coagulation of the medium**

Before loading, heat the inspissator to 80^{oc} to quicken the build-up of the temperature. Place the bottles in a slanted position in the inspissator and coagulate the medium for 45 minutes at 80^{oc}-85^{oc} (since the medium has been prepared with sterile precautions this heating is to solidify the medium, not to sterilize it). Heating for a second or third time has a detrimental effect on the quality of the medium.

The quality of egg media deteriorates when coagulation is done at too high a temperature or for too long. Discoloration of the coagulated medium may be due to excessive temperature. The appearance of little holes or bubbles on the surface of the medium also indicates faulty coagulation procedures. Poor quality media should be discarded

f). Sterility cheek: After inspissations, the whole media batch or a representative sample of culture bottles should be incubated at 35^{oc}-37^{oc} for 24 hours as a check of sterility.

j).Storage: the LJ medium should be dated and stored in the refrigerator and can keep for several weeks if the caps are tightly closed to prevent drying out of the medium. For optimal isolation from specimens, LJ medium should not be older than 4 weeks.

4.1. **Sensitivity testing of isolates for PNB.**

Serious activities were performed to test the sensitivity of isolates for PNB by seeding a 1/10.000 dilution of a suspension of *Mycobacterium tuberculosis* calibrated to McFarland No 1. (Equivalent to a bacterial suspension containing 1 mg/ml of tubercle bacilli)

- Prepare a McFarland No 1 suspension with a *M. tuberculosis* reference strain.
- Dilute the suspension with 10-fold dilutions to the 10^{-4} dilution.
- Five tubes of a previous batch of medium and 5 tubes of the new batch of medium are inoculated with 0.2 ml of the 10^{-4} diluted suspension.
- Incubate at 36°C+/- 1°C
- If the number of colonies obtained on the recently prepared or purchased batch is significantly lower than on reference batch of medium, the sensitivity of the new medium, whether prepared or purchased, is not adequate.

This register allows the identification and the elimination of deficient media batches. In the case of egg-based media, 20 days of incubation are usually enough to determine whether the sensitivity of the batch is satisfactory. If it is not, negative culture results obtained with tubes inoculated with the deficient medium will be invalidated and these cultures will be repeated. Media batches that are not homogeneous or contaminated, those that were exposed to high temperatures of inspissations as well as those showing low sensitivity, should never be used and should be discarded without delay.

3.2. **Reading: Solid media**:

- Make sure that cultures are checked at regular intervals:
- At 3 days of incubation to detect and to register early contamination
- Weekly to detect growth as early as possible.
- Confirm that new specimens have been requested in those cases when the smear positive specimens turn out to be culture negative or when all inoculated tubes/vials are contaminated.

3.3. Determination of the contamination rate: The contamination rate is a valuable indicator of the efficiency of procedures used for specimen processing. It is calculated as the percentage of contaminated tubes among all inoculated tubes or vials and not as the percentage of patients.

It should be within the range 2-4% and not exceed 5%, if the Petroff decontamination method is used. When available, computer databases should be preferred to hard copies forms to register and monitor results of positive patients and culture quality indicators

5. Drug Susceptibility Testing using MGIT

Primary Drug Susceptibility Testing (SIRE

BACTEC MGIT 960 SIRE Kit for **critical concentrations** contains the following drugs in lyophilized form. Each kit contains one each of S, I, R, and E drug vial and 8 vials of MGIT 960 SIRE Supplement.

a. Drugs

- Streptomycin - approximate lyophilized drug per vial ---------- 332µg
- INH - approximate drug per vial ---------- 33.2 µg
- Rifampin – approximate drug per vial ---------- 332 µg
- Ethambutol – approximate drug per vial ----------1660 µg

b. SIRE supplement

The SIRE supplement vial differs from the MGIT Growth Supplement and contains, per liter of purified water, the following:

- Bovine albumin ---------- 50.0 g
- Dextrose---------- 20.0 g
- Catalase ---------- 0.03 g
- Oleic acid ---------- 0.6

Storage

Upon receipt, refrigerate the lyophilized drugs at 2-8ºC. Reconstitute prior to use. Once opened and reconstituted, the leftover drug solutions may be frozen in aliquots at -20ºC or lower and stored for up to 6 months or up to the date of original expiry, whichever comes sooner. Once thawed, discard the leftover and do not store or refreeze.

Procedures

Reconstitution of lyophilized drugs

Reconstitute each critical concentration drug vial with 4 ml of sterile distilled/deionized water. Mix thoroughly and make sure the drug is completely dissolved.

Addition of a drug to the medium

Add 0.1 ml (100 µL) of reconstituted drug solution into each of the labeled BACTEC MGIT 960 tubes. This will result in the following critical concentration of drugs in the medium.

- Streptomycin ---------- 1.0 µg/ml of medium
- Isoniazid ---------- 0.1 µg/ml of medium
- Rifampin ---------- 1.0 µg/ml of medium
- Ethambutol ---------- 5.0 µg/ml of medium

Preparation of the inoculum

Inoculums from the MGIT tube: It is important that the growth is within the following recommended timeframe.

• The day a MGIT tube is positive by the instrument is considered **Day 0.**

• The tube should be kept incubated for at least one more days (**Day 1**) before being used for the susceptibility testing (may be incubated in a separate incubator at 37ºC + 1ºC).

• A positive tube may be used for drug susceptibility testing up to and including the fifth day (**Day 5**) after it becomes instrument positive. A tube that has been positive for more than 5 days should be subcultured in a fresh MGIT tube supplemented with

MGIT 960 Growth Supplement and should be tested in a MGIT 960 instrument until it is positive. Use this tube from one to five days of instrument positivity as described above.

• If growth in a tube is on **Day 1** or **Day 2**, mix well (vortex) to break up clumps.

Leave the tube undisturbed for about 5-10 minutes to let big clumps settle on the bottom. Use the supernatant undiluted for inoculation of the drug set.

• If growth is on **Day 3, 4, or 5**, mix well to break up the clumps. Let the large clumps settle for 5-10 minutes and then dilute 1.0 ml of the positive broth with 4.0 ml of sterile saline. This will be a 1:5 dilution. Use this well mixed diluted culture for inoculation.

Inoculation and incubation

• Label 5 MGIT tubes for each test culture. Label one for GC (growth control, without drug), one for STR, one for INH, one for RIF, and one for EMB.

• Aseptically add 0.8 ml of BACTEC 960 SIRE Supplement to each of the MGIT tubes. Use only MGIT SIRE Supplement and not MGIT Growth Supplement.

• Aseptically add 0.1 ml (100 microliter) or properly reconstituted STR drug in the

STR labeled tube. Similarly, add other drugs in the other labeled tubes. It is important to add the correct amount of drug to each tube. If possible, use a well calibrated micropipette for each addition. Use a separate pipette or micropipette tip for each drug. Do not add any drug to the GC tube.

Drug	**Concentration of drug after reconstitution**	**Volume added to MGIT tube**	**Final concentration in MGIT tube**
STR	83 µl/ml	100 ml	1.0µl/ml
INH	8.3 µl/ml	100 ml	0.1µl/ml
RIF	83 µl/ml	100 ml	1.0µl/ml
EMB	415 µl/ml	100 ml	5.0 µl/ml

* The drugs should be reconstituted using 4 ml sterile deionized or distilled water to achieve the indicated concentrations. Calculations of the dilution factor for MGIT medium: 7.0 ml of medium + 0.8 ml of SIRE Supplement + 0.5 ml of inoculum = 8.3 ml. Addition of 0.1 ml of the drug solution in 8.3 ml of the medium = 1:83 dilution.

• Aseptically add 0.5 ml of the well-mixed culture suspension (inoculum) into each of the drug containing tubes using a pipette. Do not add to the control.

• For the control, first dilute the test culture suspension 1:100 by adding 0.1 ml of the test culture suspension to 10.0 ml of sterile saline. Mix well by inverting the tube 5-6 times. Use this diluted suspension to add 0.5 ml into the growth control tube.

• Tighten the caps and mix the inoculated broth well by gently inverting the tube several times.

• Susceptibility test “Set Carriers” are provided in different numbers of drug combinations. For a routine SIRE test with critical concentration, a Set Carrier of five tubes is used (refer to BACTEC MGIT 960 User’s Manual for details). Place labeled tubes in the correct sequence in the set carrier (GC, STR, INH, RIF, and EMB).

• Enter the susceptibility set carrier into the BACTEC MGIT 960 instrument using the susceptibility test set entry feature. (Refer to the BACTEC MGIT 960 User's Manual, AST Instructions.) Ensure that the order of the tubes in the AST Set Carrier conforms to Set Carrier definitions. For example, GC, STR, INH, RIF, EMB for the SIRE standard testing.

• If you need to check purity of the inoculum, streak the test culture suspension onto a blood agar plate. If blood agar is not available, use chocolate agar or BHI agar. Incubate at 35 ºC + 1ºC for 48 hours and check if there is any growth. If growth appears, do not set up the susceptibility test. It may be important to establish the purity of culture before setting up susceptibility test, particularly if contamination is suspected

Waste management and other safety precautions

Used pipettes are collected inside the BSC in appropriate containers, metal or thermo resistant Plastic bins, containing disinfectant (see SOP # 23). Test tubes with bacterial suspensions, if Screw-capped tightly, can be sprayed with disinfectant and later be autoclaved as well as the pipettes. More or less open test tubes with suspensions in racks need to be tightly boxed before Transfer to the autoclave. When tubes of solid cultures are discarded in solid containers (instead of autoclavable plastic bags), water with disinfectant should be added to the bottom of containers before autoclave. Otherwise steam may not be reach cultures and tubercle bacilli may be alive after a standard autoclave cycle. Gloves and other waste may be collected in an autoclavable plastic bag, which has to be closed and autoclaved.

DECLARATION

I, the undersigned, declare that this thesis is my original work in partial fulfillment of the requirements for the degree of **MSc** in Clinical Laboratory Sciences Specialty in **Clinical Microbiology.** I understand that plagiarism will not be tolerated and all directly quoted material has been appropriately referenced.

Name of the investigator: **Kedir Abdella Abdulsemed**

Signature: _____

Date of Submission: September 9/2013

This thesis work has been submitted to Jimma University, College of Public Health and Medical Sciences, Department of Medical Laboratory Sciences and Pathology with our approval as Advisors on the MSc in Clinical Laboratory Sciences Specialty in Clinical Microbiology (at least one signature required)

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