ADDIS ABABA UNIVERSITY, FACULTY OF MEDICINE

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Drug resistance pattern of Mycobacterium tuberculosis and its association with patients` Knowledge, Attitude and Practice towards tuberculosis in Eastern Amhara Region

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Declaration

I the undersigned, declare that this thesis is my original work, has never been presented in this or any other university, and that all resources and materials used herein, have been duly acknowledged.

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Acronyms

ACSM-Advocacy, Communication, and Social Mobilization

AFB –Acid-Fast Bacilli

AIDS - Acquired Immuno Deficiency Syndrome.

BCG - Bacilli Chalmette–Guerin

BSL-2 – Bio-Safety Level two

CI- Confidence Interval

CLIS –Clinical Laboratory Information System

DOTS –Directly Observed Treatment, Short-course

DR –Drug Resistance

DST –Drug Susceptibility Testing

E –Ethambutol

EHNRI - Ethiopian Health and Nutrition Research Institute

GC- Growth Control

H –Isoniazid

HIV - Human Immunodeficiency Virus

IEC- Information, Education,and Communication

KAP – Knowledge, Attitude,and Practice

LJ- Lowenstein -Jensen Media

MDR- TB- Multi Drug Resistance Tuberculosis

MGIT - Mycobacterium Growth Indicator Tube

M.tuberculosis –Mycobacterium Tuberculosis

NALC- NaOH - N-acetyl L-cysteine- Sodium hydro oxide method

OPD- Out Patient Department

OR- Odd Ratio

PDR-TB- Poly Drug Resistance Tuberculosis

PTB –Pulmonary Tuberculosis

R –Rifampicin

S –Streptomycin

SOP –Standard Operation Procedure

SRS – Simple Random Sampling

TB - Tuberculosis

μg – Micro gram

Vs – Versus

WHO- World Health Organization

Z - Pyrazinamide

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Operational definitions

Tuberculosis: A bacterial infection caused by *Mycobacterium tuberculosis*. The disease usually affects the lungs (pulmonary) but can spread to other parts of the body in serious cases (extra-pulmonary).

Smear Positive Case: The presence of TB bacteria in a patient's sputum (sample of mucus or phlegm from a patient's respiratory tract) when examined under the microscope.

Smear negative cases: Absence of TB bacteria in a patient's sputum (sample of mucus or phlegm from a patient's respiratory tract) when examined under the microscope.

Critical concentration: Lowest concentration of an anti-tuberculosis drug in the culture medium at which growth (equal to or larger than the critical proportion) of tubercle bacilli indicates resistance of clinical significance

Critical proportion: The percentage of tubercle bacilli of the inoculums whose growth on culture media containing the critical concentration signifies clinical ineffectiveness.

Growth control: Culture yielded after inoculation of tubercle bacilli on a culture medium without any test drug in order to exhibit unrestricted growth.

Start control: A control used to control different activities during sample processing and instead sputum sample, sterile distilled water is used before sample processing start but other activities run like normal samples..

End control: A control used to control different activities during sample processing and instead sputum sample, sterile distilled water is used at the end of sample processing, other activities run like normal samples.

New case: A patient who has never had treatment of TB or has been on treatment for less than four weeks.

Relapse case: A patient declared cured or treatment completed of any form of TB in the past, but who is found to be smear positive.

Failure case: A patient while on treatment remains or becomes smear positive at the end of fifth month or later after commencing treatment.

Defaulter: A patient who has been treatment for at least four weeks and whose treatment has been interrupted for more than eight consecutive weeks or cumulative period of more than 12 weeks.

Multi-drug resistant tuberculosis: Defined as TB that is resistant at least to Isoniazid and Rifampicin ,the two most powerful first-line anti-TB drugs. Isolates that are multiple-resistant to any other combination of anti-TB drugs but not to Isoniazid and Rifampicin are not classed as MDR-TB.

Pre-XDR-TB: TB with resistance to H and R and Fluoroquinolone or a second line injectable agent (Amikacin, Kanamycin and Capreomycin), but not both.

Resistance among previously treated case: Defined as the isolation of drug resistant *M.tuberculosis* from a patient who has been treated for TB for one month or longer.

Primary drug resistance: Defined as the isolation of a drug resistant strain from a patient without a history of previous treatment.

Mutation: When a DNA gene is damaged or changed in such a way as to alter the genetic message carried by that gene.

Re-treatment case: A patient previously treated for TB undergoing treatment for a new episode, usually of bacteriologically active positive tuberculosis.

Directly Observed Treatment Short course: Watching the patient take his/her medication to ensure medications are taken in the right combination and for the correct duration.

Drug resistance tuberculosis: Confirmed through laboratory tests that show that the infecting isolates of *Mycobacterium tuberculosis* grow in vitro in the presence of one or more anti-tuberculosis drugs.

Mono-resistance: Resistance to one anti-tuberculosis drug.

Poly-resistance: Resistance to more than one anti-tuberculosis drug, other than both Isoniazid and Rifampicin.

Any resistance: Resistance to at least one anti-TB drug.

Patient delay: The length of delay between the onset of symptoms and patients first visit to health care.

Health service delay: The length of delay between health care visit and the diagnosis of tuberculosis.

Health seeking behavior: Refers to all those things humans do to prevent diseases and to detect diseases in asymptomatic stages.

Advocacy: Taking actions to help people say what they want, secure their rights, represent their interests and obtain services they need.

Communication: The exchange and flow of information and ideas from one person to another; it involves a sender transmitting an idea, information, or feeling to a receiver.

Social mobilization: Defined as a process that engages and motivates a wide range of partners and allies at national and local levels to raise awareness of and demand for a particular development objective through face-to-face dialogue.

Abstract

Background: Tuberculosis (TB) remains a serious public health problem, worsened by the emergence and spread of drug resistance particularly multi-drug resistance that threat global TB control. Data obtained from KAP survey is essential to plan, implement and evaluate Advocacy, Communication and Social Mobilization (ACSM) work.

Objectives: The aim of this study was to assess the magnitude of drug resistance pattern of *M.tuberculosis*, knowledge, perception and practice of patients` towards TB in Eastern Amhara Region, North East Ethiopia.

Methods: A cross sectional survey was conducted among new and re-treatment patients (age > 18 years old) from September 2010 to February 2011. A structure and pre-validate questionnaires was used to collect data. Primary isolation and DST were carried out on egg based LJ media using indirect proportion method. Chi-Square and multivariate logistic regression was used.

Results: Out of 230 study participants for DST, 165 were new cases while 65 were previously treated cases. From these, 66.5% of isolates were sensitive and 4.4% resistance to four first line anti-tuberculosis drugs (HRSE) while the remaining 33.5% was resistance to at least for single drug. MDR-TB was detected in 6.5 % isolates, of which 4.4% were resistance to all four first line drugs. Overall resistance to S, R, H and E was found in 27 % (62), 10 % (23), 17.8 % (41), and 6.5 % (15) respectively. Mono resistance was found in 17.4 % (40) of all isolates

Among new cases primary drug resistance for one or more drugs was observed in 23.6 % (39) cases. Primary MDR-TB was found in 3 (1.81%) cases. Similarly among previously treated cases resistance to any drug was found in 58.5 % (38) cases. MDR-TB in previously treated cases was found in 18.46 % (12) Cases; the highest being in failure cases 9.23% (6).

More over the mean and median knowledge score of respondents about PTB was 6.81 and 7 respectively. Majority (53.6%) of study subjects had poor knowledge score, feels not well informed about TB and had several misconceptions that need to be clarified. Majority (66.6%) of

study subjects heard about TB for the first time from health workers. Of study participants, 79.9% mentioned that TB transmits by respiratory droplets through coughing and sneezing and prevents by covering mouth and nose (66.6%). The four common symptoms mentioned by respondents were cough (65.6%), weight loss (33.2%), cough > = 2 weeks (32.7%) and shortness of breath (29.4%). About half of respondent not knew current free cost of TB diagnosis and treatment. Majority of respondents also worried about the disease due to it might transmits to their family, might not be cure, social interact (fear of stigma) and unable to do work. Cost (69.9 %) and difficulties in transportation (54.5 %) mentioned as the main reason for their delaines to seeking care.

Previous drug exposure and 1^{+} bacterial load independently contribute for the development of drug resistance TB strains. Similarly Illiteracy, rural residence, non-previous history of contracting TB, experiencing self treatment option, and delayed frequency of visit were independent predictor of low knowledge score.

Conclusion: Drug resistance TB particularly MDR-TB is an emerging problem in new and re-treatment patients in our study area. Majority of respondents also had poor knowledge and several misconceptions that need to be clarified. Hence, it is essential to address the problems of drug resistance through establishing good TB control program including DOTS plus service and raise KAPs` of patients through establish proper IEC pathway to indicate the level of severity and to create proper awareness about its cause, transmission, prevention and availability of service.

1. Introduction

1.1. Background Information

Tuberculosis (TB) is contagious bacterial disease caused by *Mycobacterium tuberculosis* (*M.tuberculosis*), an Acid Fast Bacilli (AFB). It is spread by aerosolization of droplet nuclei bearing *M.tuberculosis* particles released from the lung of patient with cavitary pulmonary or laryngeal disease. It is a treatable and preventable disease (1 - 3).

TB is the second most common cause of death due to an infectious disease after *Human Immunodeficiency Virus*/*Acquired Immuno Deficiency Syndrom* (HIV/AIDS) and TB will still be among the 10 leading causes of global disease burden in the year 2020 (2). This might be due to poverty, HIV/AIDS and the emergence and spread of drug resistance strains of TB particularly multi drug resistance (MDR) and extensive drug resistance (XDR) tuberculosis (2).

Drug resistance tuberculosis (DR-TB) is a case of TB (usually pulmonary) execrating bacilli resistance to one or more anti-tuberculosis drugs. MDR-TB is resistance to at least Isoniazid (H) and Rifampicin (R), the main anti-tuberculosis drugs. Similarly XDR-TB is a variety of MDR-TB that is also resistant to any Fluroquinolone and one of the three injectable second line drugs (Amikacin, Kanamycin and Capreomycin). Resistance may be resistance on previously treated case, when drug resistant mutants are selected as a result of ineffective treatment or as primary resistance (new) when a patient is infected with a resistant strain (3-5).

Since anti-tuberculosis drugs are a two-edged sword, on one hand they destroy pathogenic *M.tuberculosis*, on the other hand can select for drug resistant bacteria against which those drugs are then ineffective (6). Drug resistance in *M.tuberculosis* occurs when resistant mutants naturally occurring in the Mycobacterial population are selected by inadequate or interrupted treatment with anti-tuberculosis agents. Mutations in genes encoding drug targets or drug activating enzymes, presence of point mutations and / or deletions, presence of standardized regimen, high bacterial load, presence of cavitary condition and compromised or variation in drug penetration are responsible for the emergence of drug resistance strains. Thus, strains of MDR or XDR-TB obtained from worldwide sources are not the result of a single genetic event,

but of successive events in different loci. Since chromosomal loci responsible for resistance to various drugs are not linked, the risk of a double spontaneous mutation is extremely low. Once MDR–TB has developed, there is little to stop the rapid acquisition of resistance to the remaining agents. Further progression to pre-XDR and XDR-TB becomes only a question of time (2, 6 - 9).

Rapid emergence of DR-TB was observed not only in countries that have poor and sub optimal control programs but also in developed once. This may be due to spread of those resistance strains in the population and poorly administered prisons (10). DR-TB including MDR was varies geographically and in terms of exposure episodes. Resistance among previously treated cases is more common than primary resistance. A study conducted by Berry *et al*. showed that 11.1 % of new TB cases were resistance to any drug rises to 25.1% in previously treated cases (10, 11).

In Ethiopia DR- TB, both initial and acquired were reported from different regions of the country (34 - 38). A retrospective studies from 1984 to 2001 showed that the initial resistance to H ranges from 2% to 21% and initial resistance to Streptomycin (S) ranges from 2 to 20%. MDR - TB was reported in about 1.2% of new cases and 12% of re-treatment cases. Ethambutol (E) resistance is either nil or very low (below 0.5%) (12). According to WHO 2010 report, the level of MDR- TB in Ethiopia among new TB cases is estimated at 1.6 percent and 12% for previously treated patients (13).

HIV is the strongest risk factor for developing TB disease both in latent or new *M.tuberculosis* infection. The risk of developing TB is between 20 and 37 times greater in people living with HIV than among those who do not have HIV infection (14).

Tuberculosis detection under national TB control program in Ethiopia is very low; still at 34 % as compared with World Health Organization (WHO) plan (15) which could be influenced by the accessibility of health service and patient's health-care seeking behaviors. Delayed care is closely related with patients' demographic characteristics, knowledge of TB and traditional beliefs (16). Designing of Knowledge, Attitude, and Practice (KAP) survey and implementation of Advocacy, Communication and Social Mobilization (ACSM) is an integral part of TB control. KAP survey is very important to gathers information about what respondents know about TB, what they think

about people with TB or about the health system response to TB, and what they actually do with regard to seeking care or taking other action related to TB (17).

The accurate diagnosis of DR-TB requires a positive culture of *M.tuberculosis* and drug susceptibility testing (DST) (3). Information about drug susceptibility pattern of *M.tuberculosis* isolates against first line anti-tuberculosis drugs and its association with patients` knowledge levels are an important aspect of TB control strategies. However studies conducted about drug resistance pattern of *M.tuberculosis* and KAP of patients towards TB was very limited in these study areas. Therefore the aim of the study was to determine the prevalence of drug resistance among new and re-treatment cases and to examine KAP of patients towards TB and its association with the development of drug resistance TB bacilli in Eastern Amhara Region, North East Ethiopia.

1.2. Statement of the problem

According to WHO 2010 TB report, 9.4 million were incident cases, 14 million prevalent cases, 1.3 million deaths among HIV-negative people and 0.38 million deaths among HIV-positive people. Most cases were in the South-East Asia, African and Western Pacific regions (35%, 30% and 20%) respectively. An estimated 11– 13% of incident cases were HIV-positive; the African Region accounted for approximately 80% of these cases. There were an estimated 440, 000 new MDR-TB cases in 2008, and 150, 000 deaths from MDR-TB. It was estimated that in 2009, 3.3% of all new TB cases had MDR-TB**.** The highest MDR-TB reported (28%) of new TB cases in some settings of the former Soviet Union. XDR-TB cases have been confirmed in 58 countries (1).

The global distribution of TB cases is skewed heavily toward countries with low income and emerging economies. Africa, and more specifically Sub-Saharan Africa, faces the worst TB epidemic since the advent of the antibiotic era. These occur predominantly (approximately 6 million of 8 million) in the economically most productive 15 to 49-year-old age group (2).

Factors such as delay in seeking treatment, ignorance towards the modes of spread of the disease and treatment default could contribute to the currently high caseload of TB and its mortality and morbidity (16). For TB control and prevention knowledge and behavior of not only the patient, but also the general population are the key issues which have a profound influence on the treatment seeking behavior and completion of treatment. Poor health education and awareness about TB of the patients and health care providers are one of the fundamental problems, which adversely affect the current strategy of TB control. Lack of adequate information plays a key role as one of the major barriers to treatment compliance and leads to high caseload of TB (18).

1.2.1. Tuberculosis in Ethiopia

Ethiopia ranks seventh among the world's 22 high-burden countries with TB. According to WHO 2010 Global TB Report, the country had an estimated 44,398 TB cases in 2009, with an estimated incidence and prevalence rate of 300 and 470 cases per 100,000 populations respectively. Similarly case detection was 50% for all forms of TB. Among all new TB cases

30% were smear positive, 35% smear negative, 34% extra pulmonary and ,<1% smear unknown cases. In addition among re-treatment cases 2,259 (64%) were relapse case, treatment after failure 381(11%), treatment after default 478(13%) and 56,040 had both TB and HIV co infection (13).

Ethiopia's National TB and Leprosy Control Program began to implement Directly Observed Treatment, Short-course (DOTS) strategy for TB control in 1991. While treatment is integrated into general health services and DOTS geographical coverage is 95 percent, due to the limited health infrastructure in the country, only approximately 60 to 70 percent of the population has access to DOTS services. The DOTS detection rate remains low, at 34 percent, compared with WHO's target of 70 percent detection. The limited diagnostic capacity for TB in the country remains a challenge to improving case detection rate (15).

According to the Ministry of Health hospital statistics data, TB is one of the leading cause of morbidity and the fourth cause of hospital admission and the second cause of hospital death in Ethiopia (19).

Amhara Region has a total of 6 zonal hospitals and 9 district hospitals, 81 health centers, 508 health stations and 410 health posts. The health facility to population ratio is one hospital for 1,177,933 and one bed for 11,051 persons. Health service coverage in the region is still 51.8% and a significant proportion of the population still lives beyond catchment areas of even peripheral health institutions (19).

Incontrast with rapid expansion of health facilities in Amhara Region the prevalence of DR-TB and KAP of patients towards TB in Amhara region in general, particularly in Eastern Amhara Region is still unknown. Therefore analysis of drug resistance pattern of *M.tuberculosis* and its association with patient's knowledge level towards TB in this Region will provide information on the current drug susceptibility pattern and KAP of patients towards TB. The information obtained could help to establish proper interventions for TB prevention and control, to increase case detection and treatment compliance.

2. Literature review

The emergence of resistance to anti-tuberculosis drugs particularly MDR-TB has become a major public health problem in a number of countries and an obstacle to effective global TB control. Nearly half a million cases of MDR-TB emerge every year as a result of under-investment in basic activities to control TB, poor management of the supply and quality of anti-tuberculosis drugs, improper treatment of TB patients and transmission of the disease in congregate settings (20). Similarly WHO`s 2010 report showed that the proportion of MDR-TB among new TB cases globally ranges from 0% to 28.3% and the proportion of MDR-TB among previously treated TB cases reported globally ranges from 0% to 61.6% (21).

Aamer *et al*. in Pakistan assessed the prevalence of MDR-TB and the result revealed that resistance to single drug was noted in 23 (7.93%), majority of which were against H (7) and S (7). Forty four isolates showed resistance to two drugs: H plus R was highest (33), followed by H plus S (3) and R plus S (3). Thirteen strains (4.48%) showed resistance to three drugs, mainly resistant to R, H and S (6) and R, H and E (6) (22).

Survey of drug resistance in Taiwan indicated that a total of 55.5% were resistant to one or more drugs. Single-drug resistance was observed in 15.9% of all isolates. Mono resistance to Pyrazinamide (Z) (8.0%) was most frequent, followed by H (5.1%), R (0.5%), E (1.6%), and S (0.7%). Isolates from patients aged < 25 years showed a significantly higher drug resistance rate (79.2%) compared with other age groups (23).

Primary drug resistance study conducted in Kuwait showed that among Kuwaitis, primary drug resistance to a single anti-tubercular agent was noted in 10.3%, from this 6.0% for Z and 4.3% for H. Regarding non-Kuwaitis, primary resistance was noted in 15.3%, 8.4 % were resistant to H, 6.2% to Z, 5.2% to S, 1.7% to R, and 1.5% to E. Two drug resistances were detected in 5.6%, and three-drug resistance was noted in 1.1 %. One non-Kuwaiti patient was resistant to four anti-tuberculosis agents (24).

Similarly national drug resistance surveillance in Pakistan showed that 64% were resistance to one or more agents. MDR-TB was observed in 47% isolates. Primary resistance to any one or

more agent was noted in 39% and resistance among previously treated cases in 79% isolates. MDR-TB on new case occurred in 10% strains while 69% in previously treated cases. And conclude that MDR-TB was mainly associated with previous anti-tuberculosis treatment (25).

A study conducted by Shamaei *et al*. in Iran among drug users, the result showed that 14.1% were found to have any resistance to anti-TB drugs, and 1.1% individuals had MDR –TB (26). Moreover Shamaei *et.al* showed that MDR –TB was identified in 2.8% of the new TB cases. Any resistance was detected in 41.6%. Drug-resistant TB had a statistically significant association with Afghan ethnicity, age>65 years, and the type of disease (re-treatment versus vs new TB case) significantly. Drug resistance trends showed a significant increase in resistance to any anti-TB agent, to Isoniazid, and to Streptomycin in new cases, and to all of the first-line anti-TB drugs in re-treatment patients (27).

A retrospective study in Saudi Arabia by Khan *et al.* described that 29.7% of *M.tuberculosis* isolates were resistant to one or more anti-tuberculosis drugs and 20% were MDR-TB. Resistance was most common to H (28.7%), followed by S (22.8%) and R (20.8%). Resistance to Z and E was 7.9% and 6.9% respectively (28).

In Turkey Surucuoglu *et al.* showed that any drug resistance and MDR-TB rates were 21.1% and 7.3%, higher in males (53% and 9%) than in females (22% and 1%) respectively. Drug resistance was significantly higher in previous exposed cases than new cases. It is also associated with treatment failure significantly. The prevalence of MDR-TB was significantly higher in the previously exposed cases (22.4%) than in the new cases (4.4%) significantly. The prevalence of any drug resistance and MDR-TB was significantly higher in those with treatment failure than in patients with treatment success (29).

Green *et al*. using Mycobacterium Growth Indicator Tube (MGIT) 960 in South Africa showed that 30.2% strains were resistant to one or more drugs. Resistance to one drug ranged from 1.4% for E to 17.7% for R. The prevalence of MDR-TB ranged from 6.7% for three drugs to 34% for four drugs, with significant predictors being patients' age groups of 25-54 years and >55 years significantly. The study conclude that a high level (58.4%) of MDR-TB was occurred among re-treated cases (30). Similarly pulmonary tuberculosis (PTB) drug resistance profile study in

Nigeria using Lowenstein -Jensen Media (LJ) medium by the proportion method, the result showed that MDR-TB was obtained on 4, and 18% of the new and follow-up cases respectively. Mono-resistance was found in 15% of the cases (31).

Moreover Phyu *et al*. using MGIT 960 in Myanmar indicated that 29.3% new patients and 45.9% previously treated patients were resistant to at least one of the anti-TB drugs. Resistance to H (22.0% vs 40.8%: new vs previously treated patients) and to > or =2 drugs (17.8% vs 29.6%: new vs previously treated patients respectively) was common. MDR-TB among new and previously treated patients was 4.2% and 18.4% respectively. H-resistant and MDR-TB cases were more likely to have taken anti-TB drugs > or = one month previously (32).

Drug resistance study on previously treated cases in Ethiopia by Meskel *et al*. showed that *M.tuberculosis* isolates resistance to at least one drug was observed in 53.6% while 26.2% of the isolates were MDR-TB and conclude that MDR-TB is an emerging problem among re-treatment cases of pulmonary TB in Addis Ababa (33). Asmamaw *et al*. Using Middle brook 7H10 media also indicated that 21.4% were resistant to at least one drug, single drug resistance to S was observed in 16.2%, to H in 13.3%, to R in 1.2% and to E in 3.5% of the isolates. The prevalence of resistance to at least one drug was 15.7 % and 23.7% among patients with and without HIV co-infection respectively ($p > 0.05$). Only one patient (0.6%) had MDR-TB strain (34).

Similarly primary drug resistance by Demisse *et al*. in Addis Ababa indicated that overall primary drug resistance was found to be 15.6%; Primary resistance to two or more drugs was 7.2%. The highest rate of primary resistance was to S (10.2%) followed by H (8.4%). Resistance to R was low (1.8%) and to E nil. MDR-TB in combinations with R was low (35).

Abate *et al*. in Addis Ababa among retreatment cases using BACTEC 960 method revealed that about 50% of the strains were resistant to one or more of the first-line drugs and 12% of the strains were MDR –TB and the previous treatment with R was the most important predictor of MDR-TB (36). Moreover Kassue *et al*. in Addis Ababa among smear positive and negative cases using LJ media depicted that drug resistance to any drug was observed in 29.8% and Streptomycin mono resistance in 24.3% (37).

Similarly globally there are many researches that assessed KAP survey among population and special sub groups like a study conducted in Iraq to understand and measure the attitudes, practice and level of knowledge among a general population, the result showed that there was strong community attitude regarding TB as a stigma mainly among women and toward avoiding patients with TB. The disease had a bad impact on social relations with other family members and lack of proper knowledge among a general population (38).

Another cross-sectional study was conducted on patients who attended the TB clinic for their medication in Tanzania and the result showed that discrimination against TB patients by relatives and friends is likely to hinder positive health seeking behaviour and impede control of disease (39). Shetty *et al*. also assessed survey regarding TB among Somalian immigrant and result showed that men having a significantly higher knowledge than women (40).

In Vietnames, Hoa NP *et al.* assessed knowledge of TB among men and women with a cough more than three weeks and their health seeking practice, result depicted that a large proportion of individuals with a cough for more than three weeks had limited knowledge of the causes, transmission modes, symptoms, and curability of TB. Men had a significantly higher knowledge score than women (3.04 vs 2.55). Better knowledge was significantly related to seeking healthcare on health facilities. More men than women did not take any health care action at all (41).

Hoa NP *et al*. also indicated that men had significantly higher knowledge score than women (4.8 vs. 4.0), and gender, occupation, economic status, education, and sources of information were significantly associated with level of TB knowledge. The most common sources of information included television (64.6%), friends/relatives (42.7%) and sources of information differed between men and women (42). Similarly study conducted on rural to urban migrants of china showed that low awareness, poor knowledge about TB; low financial capacity to pay for care and diagnostic were factors pose barriers to TB diagnosis (43).

A study conducted in Pakistan to assessed patients` knowledge, attitude and misconception regarding TB, the result showed that inhaled droplets were recognized as the common source of infection but eating contaminated food (47.6%), use of blood products (32.9%) and inheritance

(27%) were important modes of transmission. The four most commonly recognized symptoms were cough (83.5%), fever (54.7%), chest pain (24.7%) and bloody sputum (24.7%). Of respondents 57% explained separating dishes as preventing means of the spread of TB. Health care worker were the main source of information about TB in 127 (75%) cases (44).

Mushtaq *et al*. in Pakistan that assessed community KAP about TB, the result depicted that 42% of the surveyed population had good knowledge, which was associated with better education, high income and good housing and majority (82.2%) knew about correct treatment, but less than half (48.8%) were aware that diagnosis and treatment were free. Intended health-seeking behavior was determined by better education, good housing and good knowledge about TB. Television (69.4%) and health workers (43.6%) were the main sources of information (45). Mushtaq *et al*. also showed respondents' knowledge regarding TB was deficient in all aspects, particularly in the rural areas and less than half of respondents were aware of the diagnosis and treatment free cost of TB. Television and health workers were the main sources for TB related information in both urban as well as the rural areas (46).

A systematic review in Norway showed that rural residence, low awareness of TB, incomprehensive beliefs; self-treatment; and stigma, low educational level, old age; poverty; female sex, low access (geographical or socio-psychological barriers); coexistence of chronic cough, initial visitation of a government low-level healthcare facility, and traditional healer were important factors associated with diagnostic delay for TB (47).

A cross sectional study conducted in Croatia among patients in out-patient setting, the result showed that low knowledge score was associated with the youngest age group (18-29 years) and education (< 12 years) significantly. There were several misconceptions that need to be clarified. And conclude that educational background and age groups of respondents were important determinants of TB knowledge (48).

Inaddition Mengistu *et al*. in Tigray assessed community KAP and reported that most respondents (86.8%) had heard about PTB from health professionals (41%), friends (34.3%), relatives (14.5%), public radio (3.2%) and (0.6%) television for the first time. Exposure to cold (37%), germ/virus (9.6%), malnutrition (4.5%) and poor sanitation (4.7%) were regarded as

primary causes of PTB. Most (67.9%) believe it could be transmitted from diseased to healthy person by cough (62.5%), drinking raw milk (35.7%), eating together (40.3%), sleeping together (54.4%), touching (30.8%) and inherited from a parent to a child (33.2%). Coughing, loss of weight and night sweats was mentioned as symptoms/signs of PTB by 71.5%, 66.5% and 33.3% of respondents respectively. 69.4% stated that the disease is curable with modern therapy. The study conclude that female respondents, illiterates and rural residents were more likely to have a low level of knowledge score (49).

Demissie *et al*. in Addis Ababa assessed patient and health service delay in the diagnosis of PTB and depicted that the time before diagnosis in TB patients was long and appears to be associated with patient inadequate knowledge of TB treatment and distance to the health facilities (50). MoreoverYimer *et al*. in Amhara region assessed health seeking practice on rural community and showed that a previous history of TB and chronic cough were more likely to visit a medical health provider compared to those with a shorter duration of cough and with no history of TB (51).

2.1. Significance of the study

The emergence and spread of DR particularly MDR-TB threat global TB control both in developed and developing countries but the burden is high in countries having sub optimal and poor control programs especially in developing ones including Ethiopia (2, 10). Hence information on the susceptibility patterns of *M.tuberculosis* isolates against anti-TB drugs is an important aspect of TB control. DST of anti-TB drug resistance helps in detection, monitoring and prevention of transmission of DR and MDR strains. DST result could also be an input to the control program and improving the quality of TB control.

In line with this assessment of KAP of patients` towards TB were very important to gather information for planning public health programs, problem identification and planning intervention based on the gaps in their KAP towards TB. It can also identify cultural beliefs, or behavioral patterns that may facilitate understanding and action, as well as pose problems or create barriers for TB control efforts, and attitudes that are commonly held, to establish baseline levels and measure change that results from interventions, and for ACSM (17) work. Inaddition it will be used to establish proper information, education and communication (IEC) path way to indicate the level of severity and to create proper awareness about its cause, transmission, and prevention nature of the disease (17). This collaborative effect will be result in to increase case detection, treatment compliance and to reduce transmission and development of drug resistance strains in the community by designing proper interventions.

Therefore assessment of drug resistance pattern of *M.tuberculosis* and its association with patient's knowledge level towards TB in Eastern Amhara Region will provide information on the current drug susceptibility pattern and KAP of patients` towards TB. The information obtained could help to establish proper interventions for TB prevention and control, to increase case detection and treatment compliance.

3. Objectives

3.1. General objective

- To assess the magnitude of drug resistance pattern of *M.tuberculosis*, knowledge, perception and practice of patients towards TB in Eastern Amhara Region, North East Ethiopia.

3.2. Specific objectives

- To determine the prevalence of drug resistance pattern of *M.tuberculosis* for first line anti -tuberculosis drugs.
- To determine the prevalence of MDR-TB in Eastern Amhara Region.
- To compare drug resistance pattern of *M.tuberculosis* among new, and re-treatment cases
- To determine the KAP of patients towards TB and association of knowledge level with development of drug resistance.

3.3. Hypothesis

- Development of drug resistance TB bacilli may not have any association with patients` knowledge level and previous exposure to anti-TB drug.

4. Material and methods

4.1. Study setting

Eastern Amhara Region includes South Wollo, North Wollo , Oromia Zone, Semien Shewa and Wagemera Zones and according to 2005 Central Statistics' South Wollo has an estimated total population of 2,942,886, of which 1,446,752 were males and 1,496,134 were females, with an estimated area of 16,956.06 square kilometers, with population density of 173.56 people per square kilometer (52).

North Wollo Zone has also an estimated total population of 1,731,849, of which 864,907 were males and 866,942 were females. With an estimated area of 16,400.98 square kilometers, has an estimated population density of 105.59 people per square kilometer. Again Oromia Zone has an estimated total population of 639,107, of which 319,521 were males and 319,586 were females. With an estimated area of 4,434.53 square kilometers, has an estimated population density of 144.12 people per square kilometer (52).

Similarly Wagemra Zone has an estimated total population of 375,600, of which 187,915 were males and 187,685 were females. With an estimated area of 8,329.70 square kilometers, has an estimated population density of 45.09 people per square kilometer. Semien Shewa Zone has also an estimated total population of 2,159,301, of which 1,080,266 were males and 1,079,035 were females; 255,275 or 11.8% of its population are urban dwellers. With an estimated area of 16,070.23 square kilometers, Semien Shewa has an estimated population density of 134.37 people per square kilometer (52).

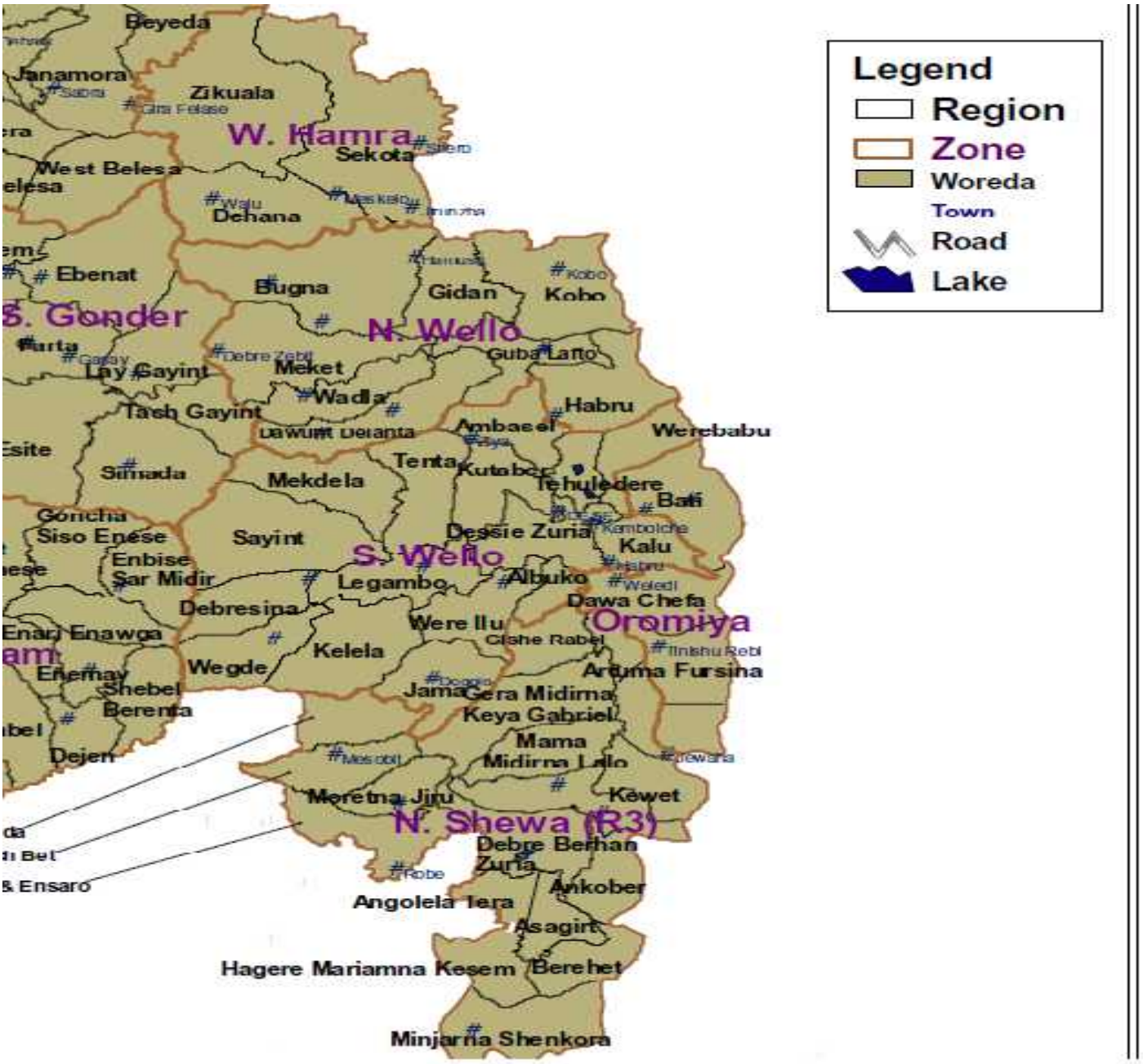

Figure 1: Political map of Eastern Amhara Region, North East Ethiopia, February 2011.

4.2. Study design and period

A cross-sectional study was conducted at selected health facilities in Eastern Amhara Region from September 2010 to February 2011.

4.3. Source population

The source population for both DST and KAP were all patients who are suspected for TB and those who are normally on follow up cases at OPD and DOTS of selected health facilities in Eastern Amhara region respectively.

4.4. Study subjects

The study subjects for DST analysis were all newly diagnosed and retreatment patients who are smear positive by Ziehl Neelson method, while for KAP not only smear positive but also smear negative cases participated in the study from OPD and DOTS of those selected health facilities.

4.5. Sampling methods and procedure for both KAP and DST

Study sites were selected (both for DST and KAP) from those health facilities based on patient flow, presence of both TB clinics and direct microscopy for AFB regularly. From those, study sites selected by simple random sampling (SRS) and from each zone four health facilities included to the study. The study sites were includes Dessie hospital, Combolecha health center, Werylu health center, Akesta hospital, Ataye and Tarmabire health center, Bati and Kemissa health center, Senebeta and Chefa health center, Woldia hospital, Kobo health center, Mersa and Hara health centers, Tefera Hailu memorial hospital, Sekota health center, Zequala and Asketema health centers, Debreberhan hospital and Shewarobit health centre. Patients who visited those health facilities, voluntary and give their consent to participate were included in the study.

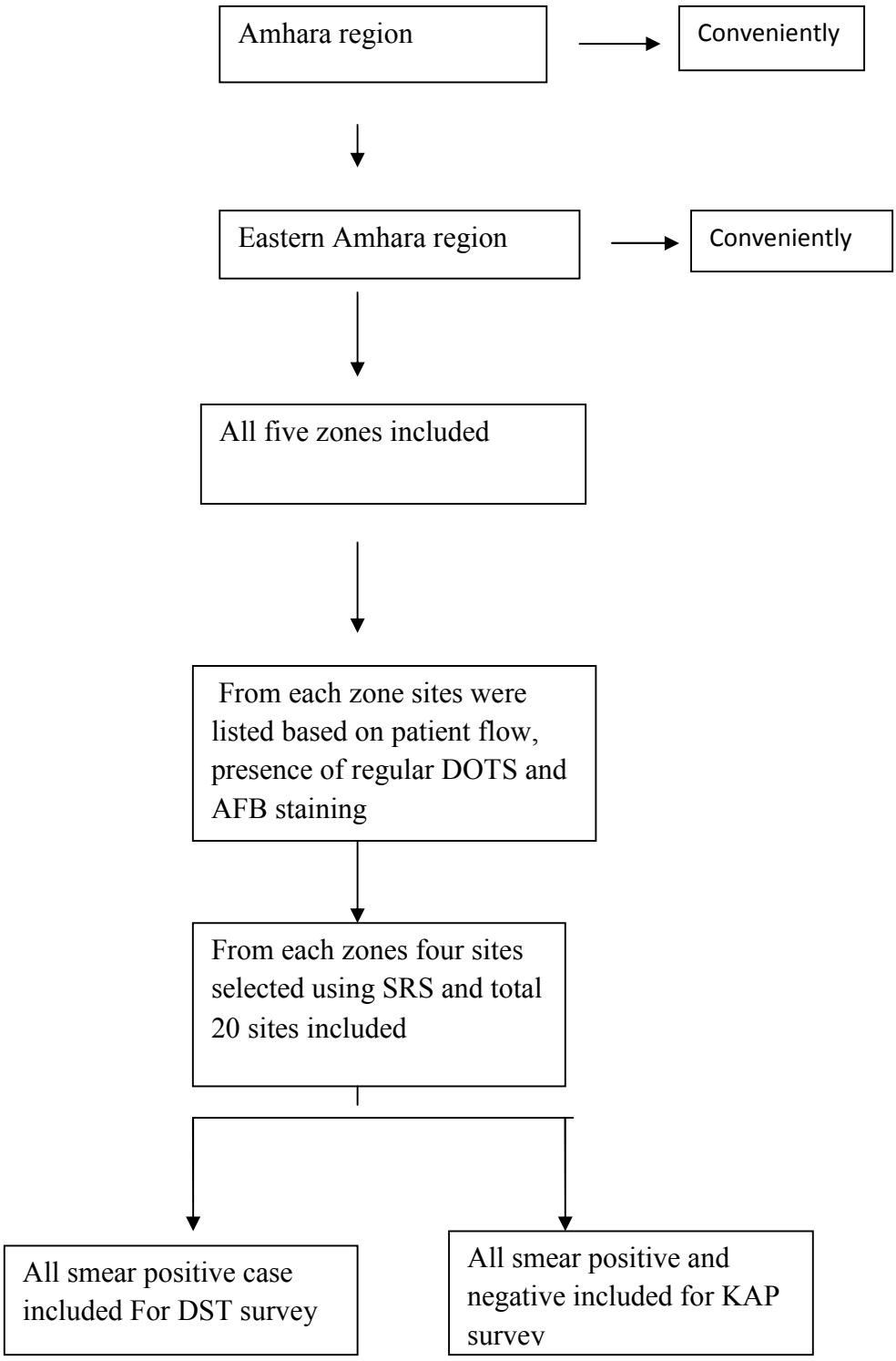

Fig. 2: Schematic presentation of sampling procedure, February 2011.

4.6 .Sample size determination for DST

The required sample size of the study population was determined using the formula for single population proportion. According to WHO 2009 global TB report, the prevalence of smear positive *M. tuberculosis* in Ethiopia is 286/100,000 (15). So the sample size was:

$$N = \frac{(Z\alpha/2)^2 * (1-p) * (p)}{(d)^2}$$, Where N = minimum sample size

Zα/2 = 1.96 at 95% Confidence Intervals (CI)

P = National smear positive TB prevalence

d= margin of error 0.05 at 95% CI

N = $(1.96)^2$ * (1-0.286) * (0.286) divide by $(0.05)^2$,

N = 313, with 10 % contingency, N =343

4.7. Sampling size determination for KAP study

Since similar study sites were used for both DST and KAP analysis and the prevalence not specifically known for KAP, we use 50 % to calculate the sample size, so the sample size was :

$$N = \frac{(Z\alpha/2)^2 * (1-p) * (p)}{(d)\ 2}$$, Where N = minimum sample size

Zα/2 = 1.96 at 95% Confidence Intervals (CI)

P = 50 % % prevalence d= margin of error 0.05 at 95% CI

$$N = \frac{(1.96)^2 * (1-0.5) * (0.5)}{(0.05)^2}$$

N =384, with 10 % contingency N =422

N.B: Eventhough 422 study subjects should included for both DST and KAP survey, only 230 smear positive cases was included for DST analysis due to presence of national TB surveillance and TB laboratory was busy and limitation of budget.

4.8. Patient inclusion and exclusion criteria

Inclusion and exclusion criteria for DST: those new and re-treatment patients who are normally smear positive and patient's age greater than or equal to 18 years were included in the study but those patients who were extra pulmonary, patients age less than 18 and confirmed smear negative pulmonary case were excluded.

Inclusion and exclusion criteria for KAP: All smear positive and negative case PTB, patient's age greater than or equal to 18 years (new and re-treatment) were included but patients who were extra pulmonary and their age less than 18 years were excluded in the study subjects.

4.9. Study variables

Dependent variable for DST and KAP: the result of drug susceptibility pattern and KAP of patient's.

Independent variables for DST: were previous anti-tuberculosis drug exposure, age, occupation, sex, education, load of bacilli, socio demographic status and knowledge of TB.

Independent variables for KAP: were good awareness and practice towards tuberculosis, age, sex, socio demographic status, education, availability for source of information, previous contracting of TB, occupation, sputum smear status for AFB.

4.10. Data collection for KAP study

A questionnaire-based survey on KAP of patients` about TB was conducted at selected health facilities of September 2010 to February 2011. A structure and pre-tasted questionnaire used to collect data. The questionnaire also translated into Amharic version. Onsite training gave for

interviewers and supervisors for two days. Crosschecking conducted in sample facilities for consistency. Verbal consent was obtained from each respondent. Questionnaires include information on the socio demographic characteristics of the respondent, knowledge, attitudes, and health seeking practice towards TB that stated on the annex part adopt from WHO ACSM questionnaires with some modifications. Prior to the administration of the questionnaire the subjects were brief on the objectives of the study.

For each TB knowledge questions a score of one was given to a correct answer while zero score for incorrect and do not know responses. Questions on the knowledge part were rated and a total score was obtained. Then median score was computed. Therefore those with a total score equal to or below the median were classified as having poor knowledge, while those above the median considered having good knowledge. For attitude and practice section frequency table were computed and practice section associated with knowledge level.

4.11. Sputum collection for drug susceptibility testing

Sputum samples were collected from patients who had smear positive result using Zihel Neelson method. During collection three consecutive sputum samples at least 5-10 ml collected in clean, sterile, leak-proof, screw caped wide-mouth, disposable containers (53). At the time of sample collection, a structured questionnaire used to collect data such as Age, Sex, and other clinical presentation and history of patients.

4.12. Sample transportation

Sputum samples transported according to standard protocol that is the container sealed and packaged, labeled, and transported in water-tight container (Ice box) (53). During transportation, each cup containing sputum samples covered by plastic separately inside ice box that obtained from Ethiopian Health and Nutrition Research Institute (EHNRI) TB laboratory.

4.13. Sample digestion and decontamination

The sputum sample analyze for *M.tuberculosis* was decontaminated by N-acetyl L-cysteine-sodium hydro oxide method (NALC-NaOH) methods as stated in the Annex part (53).

4.14. Direct smear preparation

Smear was prepared by taking a small portion of the purulent part (select the cheesy, necrotic, bloody –tinged ticles) with an applicator stick, and smeared on a microscope slide, which was then air dried. Similarly after culturing the growth confirmed for the presence of Acid Fast Bacilli using standard procedure proceeds according to the Zihel Nelseen method stated in the Annex part (53).

4.15. Inoculation and Incubation of Culture media

Primarily condense moisture observes at the bottom of culture medium slants were removed before inoculation. Inoculation for primary isolation, identification, and susceptibility testing of *M.tuberculosis* run at Bio-safety Level two (BSL-2) cabinet using pipettes. Each slope inoculates 100 µl of the centrifuged sediment and distributed over the surface. Two slopes of LJ medium were inoculated per specimen, but preparation of inoculums for susceptibility testing was according to the McFarland standards of inoculums preparation procedure stated in the annex part (53).

All cultures incubate at 35°-37°C until growth is observed or discarded as negative after eight and six weeks for primary isolation and susceptibility testing respectively. Inoculate media preferably be incubated in a slant position for at least 24 hours to ensure even distribution of inoculums. Thereafter, if incubator space is needed bottles placed upright. Tops should be tightened to minimize evaporation and drying of media (53).

4.16. Drug susceptibility testing methods

Egg based LJ medium was used for primary isolation and drug susceptibility testing. An indirect proportion method used for susceptibility testing. The amount of final drug concentration in LJ medium for H, R, S, and E was 0.2, 40.0, 4.0, and 2.0 microgram (µg) respectively (53).

4.17. Result Interpretation

4.17.1. Direct microscopy reporting

Smear was examined using a light/electrical microscope scanning 100 oil immersion fields before reporting a smear as negative or positive. Acids Fast Bacilli in specimens were red rods shaped, 1 to 10 micro meter long and 0.2 to 0.6 micrometer wide but they also appear coccoide or filamentous (long, slender, even branching) but back ground and other cells stained blue (53).

4.17.2. Culture reading

Egg based LJ was examined for growth twice a week for the first four weeks starting on day 3 post inoculation, thereafter once a week until the eighth week. All specimens showing growth in culture confirmed as AFB by smear microscopy of the colonies and reported immediately as "culture positive for *Mycobacterium* pending identification". *M.tuberculosis* bacilli, in primary isolation, they hardly show any visible growth during the first week of culture. On egg-based media they produce characteristic non-pigmented colonies, with a general rough, white creamy and dry appearance simulating breadcrumbs. Contaminated cultures and rapidly growing *Mycobacterium* (colonies yielded in less than 7 days) removed and repeated sample processing from the sediment (53).

M.tuberculosis colonies were well developed within 3 to 4 weeks as white creamy appearance on LJ media and results reported immediately after detection and cultures were kept up to 8 weeks if no growth is detected at weekly examination (53).

4.17.3. Biochemical testing

The final species identification of *M.tuberculosis* based on characteristics such as slow growth, colony morphology, and biochemical tests. An initial identification as *M.tuberculosis* defined on AFB bacilli from slow growing, non-pigmented colonies that was niacin positive, display nitratase activity and catalase negative at 68 ^{o}c (53).

4.17.4. Drug susceptibility testing

A strain considered “susceptible” if no growth or considerably less than 1% growth is detected on the test medium containing the critical concentration of the corresponding drug compared to the growth control with 1 % inoculums. A strain considered “resistant” if the growth on the culture medium containing the critical concentration of the corresponding drug shows more growth than the control with the 1% inoculums. If any contamination and borderline result occurred, the sample processes again from the sediment (53).

4.18. Quality control

Quality control for sample collection, transportation, digestion /decontamination, microscopy, culture and drug susceptibility testing were employed from EHNRI-TB laboratory procedures.

The sputum specimen was thick, mucoid and purulent, volume not less than 2 ml. All reagents, antibiotics, media (PH, colour, consistency) and other accessories prepared, used and stored in accordance with Standard Operation Procedure (SOP) used at EHNRI TB laboratory. For the quality of primary isolation both end and start control were used likewise standardized suspension H37Rv for DST. Ten percent of the target population asked to answer the questionnaires for validation. The final questionnaire revised based on the pre-validate questionnaires and preliminary data. The questionnaires also translated to Amharic version.

4.19. Statistical analysis

Data entry and cleaning was done by a trained encoder using EPI-INFO (Center for Disease Control and Prevention Atlanta, GA) version 3.5.1 and further statistical analyses made in SPSS (Statistical Package for Social Science) version 16. Descriptive statistics computed and Chi-Square used to assess the associations of different variables with development of drug resistance, patient’s KAP and patient’s knowledge with development of drug resistance. $P < 0.05$ was statistically significance. Finally multivariate analysis using logistic regression model computed to know factors independently influence dependent variables (DST and KAP).

4.20. Ethical clearance

The research proposal was evaluated by the research and ethics committee of School of Medical Laboratory Science and reviewed and cleared by Institution of Review Board (IRB) before the start of fieldwork.

Addis Ababa University/Medical Faculty, School of Medical Laboratory Science wrote official letter of co-operation to those administrative zone health departments and EHNRI for the purpose of sample collection and laboratory testing. Each administrative zone health department also wrote co-operation letters to those selected administrative woredas health office and those woreda administration offices sent a letter of co–operation to those selected health facilities. During data collection there were a high degree of confidentiality and informed consent also obtained from each study subject, no name and other identifier on the questionnaire. At the end of the study, one copy of the study finding was submitted to each zones health department and patients who developed MDR-TB are announce for the concerned body and treatment will be given accordingly.

4.21. Dissemination of results

The findings of this study will be presented to School of Medical Laboratory Science and the result will be disseminated to the administrative zone in the study area. The findings will also disseminated to different organizations (governmental and non-governmental) that had contribution to improve and preventing the wide spread of *M.tuberculosis*. Findings will present in different seminars and workshops to disseminate and it may also be submitted to journal for possible publication.

5. Results

5.1. Drug susceptibility testing

5.1.1. Socio demographic characteristics

A total of 230 smear positive respondents were participated in this study. Of these, 137(59.8 %) were males. The mean age of the respondents was 32.8 ranging from 18 to 73 years old. Majority 75.7% (174), 57.8 %(261) were married and rural residence respectively. Farming was the means of livelihood for most. Similarly 44.3 % (102) were illiterate. Of the respondents 53.9% (128) were Amhara and 50.4 %(206) christian (Table 1). Total number of sputum sample taken from North Wollo was 47. Similarly 48 from South Wollo, 51 from North Shoae, 45 from Oromia and 39 from Waghimera Zone.

Table 1: Socio –demographic characteristics of smear positive cases in Eastern Amhara Region, North East Ethiopia, February 2011.

Variables	Frequency	Percent (%)
Age		
18-30	128	55.7
31-40	51	22.2
41-50	31	13.5
>=51	20	8.7
Sex		
Male	137	59.8
Female	93	40.4
Religion		
Muslim	114	49.6

Christian	116	50.4
Education		
Elementary &junior	57	24.8
High school	36	15.7
College	13	5.7
Religious school	11	4.8
Literacy class	11	4.8
Illiterate	102	44.3
Residence		
Urban	97	42.2
Rural	133	57.8
Ethnicity		
Amhara	124	53.9
Oromo	44	19.1
Tigray	14	6.1
Agew	40	17.4
Other	8	3.5
Marital status		
Married	174	75.7
Unmarried	56	24.3
Occupation		

Farmer	117	50.9
Governmental employee	10	4.3
Local trader	34	14.8
Student	17	7.4
Daily labour	42	18.3
Other	10	4.3

5.1.2. Clinical symptoms and contact history

The majority of patients came with a combination of symptoms. The most frequently reported symptom was chronic cough 199 (86.5%), production of sputum 180 (78.3%), night sweet 148 (64.3 %), fatigue/tiredness 146(63.3 %), shortness of breath 130(56.5%), unexplained weight loss 115(50 %) and fever 55 (23.9%). Of study participants, 25 (10.9%) had contact history with known positive PTB cases and 21 (9.1 %) with suscepected TB case.

5.1.3. *Mycobacterium tuberculosis* drug resistance profile of study subjects

Out of 230 study subjects, 165 (71.7%) were new cases, while 65 (28.3%) were previously treated cases. Of all isolates, 66.5 % (153) were sensitive and 4.4% (10) resistances to four first lines anti tuberculosis drugs (HRSE) while the remaining 33.5(77) % were resistance to at least single drugs. MDR-TB was found in 6.5% (15) isolates; of this 4.4 % (10) MDR-TB cases were resistant to four first line anti-tuberculosis drugs (SHRE). Overall resistance to S found in 27 % (62), to R in 10 % (23), to H in 17.8 % (41), and to E in 6.5 % (15) (Table 2).

Mono resistance found in 17.4 % (40) of isolates, of this S and H constitute highest proportion and accounted for 65 %(26) and 30 % (12) respectively. Of all isolates, 9.6 % (22) were Poly resistance, 8.7 % was resistance for two drugs while 3.04% was resistance to three drugs. Combination of HS and SR constituted majority of cases among two drug resistance cases and

accounted for 55 % (11) and 30 % (6) respectively. Majority of three drug resistance cases was occurred by a combination of HSR 57.4% (4) (Table 2).

Table 2: Dug resistance profile of *M.tuberculosis* isolates in Eastern Amhara Region, North East Ethiopia, February 2011.

Resistance pattern	Frequency	Percent (%)
Susceptible to all	153	66.5
Resistance to at least:		
One drug	77	33.5
One drug only	40	17.4
H	12	5.2
S	26	11.3
R	2	0.86
E	-	-
Two drugs	20	8.7
HR	1	0.43
HS	11	4.8
SE	2	0.86
SR	6	2.6
Three drug or more		
HRS	4	1.7
HSE	3	1.3
HRSE	10	4.3
Over all resistance		

H	41	17.8
S	62	27
R	23	10
E	15	6.5
MDR-TB	15	6.5

H-Isoniazid, S- Streptomycin, R- Rifampicin , E-Ethambutol, MDR- TB -resistance to at least Isoniazid and Rifampicin , "_": indicates absence of resistance

Of new cases (165), primary drug resistance for one or more drugs observed in 23.6 % (39) and 13.3 %(22) for one drugs, 7.9 %(13) for two drugs, 1.8% (3) for three drugs and 0.6% (1) for all four first line drugs. Primary MDR - TB was found in 1.8% (3) cases. Majority of single drug resistance in new cases occurred by S- 63.6% (14) and H- 31.8 % (7), while of two drug resistance cases combination of HS constituted highest proportion followed by SR. Similarly among three drug resistance cases combination of HSE (2) and HSR (1) constitute (66.7%) and (33.3%) of new cases respectively (Table 3).

Similarly of previous treated cases (65), 4.6% (3) were default, failure 40 % (26), and relapse 55.4 %(36) cases. Among treated cases resistance to any drug found in 58.5 % (38), to one drug in 27.7 % (18), for two drugs in 10.8 %(7), for three drugs in 6.2 %(4), and for four drugs in 13.9% (9). MDR-TB on previously treated cases found in 18.5% (12) and it was highest in failure case (9.23%), followed by relapse (7.7%) and defaulter case (1.54%) respectively. Majority (66.7%) of previously treated single drug resistance cases were occurred by S while of two drug resistance cases SE (14.3 %) constituted the lowest proportion. Similarly for three drugs resistance cases, HSR constituted 75% of the case (Table 3).

Table 3: Drug susceptibility pattern of *M.tuberculosis* among new and re-treatment cases in Eastern Amhara Region, North East Ethiopia, February 2011.

Resistance status	New case(n=165)		Re-treated cases (n=65)			
	No	%	No	%	Total (n=230)	
					No	%
Any resistance	39	23.63	38	58.46	77	33.48
Multi-drug resistance	3	1.81	12	18.46	15	6.52
HR	1	0.6	-	-	1	0.43
HSR	1	0.6	3	4.62	4	1.74
HSRE	1	0.6	9	13.85	10	4.35
Resistance to one drugs only	22	13.33	18	27.69	40	17.39
H	7	4.24	5	7.69	12	5.22
S	14	8.48	12	18.46	26	11.30
R	1	0.6	1	1.54	2	0.86
E	-	-	-	-	-	-
Resistance to two drugs	13	7.87	7	10.76	20	8.69
HS	7	4.24	4	6.15	11	4.78
SR	4	2.42	2	3.08	6	2.61
SE	1	0.6	1	1.54	2	0.86
HR	1	0.6	-	-	1	0.43
Resistance to three drugs	3	1.81	4	6.15	7	3.04
HSE	2	0.12	1	1.54	3	1.30
HSR	1	0.6	3	4.62	4	1.74
Sensitive to all drugs	126	76.36	27	41.53	153	66.52
Resistance to all drugs	1	0.6	9	13.85	10	4.35

Majority (58.4 %) of any resistance cases occurred in age group 18-30 years. Similarly more males were harboured drug resistance strain with the ratio of male to female cases was 1.75:1. About 59.7 % (46) of any resistance cases were reported on rural area. Inaddition 13% (10) of resistance cases had contact history with suscepected TB case. Among resistance case 48.1% had poor knowledge score (Table 4).

On univariate analysis statistically significant increment on development of drug resistance seen in previous drug exposed patients (OR = 4.547, 95% CI = 2.470 – 8.370, P-value = 0.000), 1^{+} bacterial load of (OR = 4.273, 95% CI = 1.670 – 10.936, P-value = 0.002), poor knowledge score (OR = 1.821, 95% CI = 1.046 – 3.170, P-value = 0.034). Similarly increment on development of drug resistance also observed on farmer, local trader, and students (OR = 4.00, 95% CI = 1.279 – 12.510 P-value = 0.017, OR = 6.50, 95% CI = 1.712 – 24.683 P-value = 0.006, OR =16.500, 95%CI = 3.707 – 73.436, P-value = 0.000) respectively. But sex, age group, residence, education, productive cough and cough > two weeks, MDR-TB, mono and poly resistance not had any significant association with development of drug resistance ($p > 0.05$) (Table 4). Similarly development of drug resistance not had any significant association with geographical distribution of those five administrative zones ($P > 0.05$).

Table 4: Drug susceptibility patterns of smear positive patients with different variables in Eastern Amhara Region. North East Ethiopia, February 2011.

	Any resistance					
Variables	**Total**	**Yes**	**No**	**95 % CI**	**OR**	**P-value**
Age Group						
18-30	128(55.7)[a]	45 (35.2)[a]	83(64.8)[a]		1	
31-40	51(22.2)	14 (27.5)	37 (72.5)	0.702-2.92	1.433	0.324
41-50	31(13.5)	9 (29.3)	22 (71)	0.563-3.120	1.325	0.519
>=51	20(8.7)	9 (45)	11 (55)	0.256-1.718	0.663	0.397
Sex						
Male	137(59.6)	49(35.8)	88(64.2)	0.440-1.360	0.774	0.373
Female	93(40.4)	28(30.1)	65(69.9)		1	
Education						
Literate	98(42.6)	37 (37.8)	61 (62.2)		1	
Illiterate	132(57.4)	40 (30.3)	92 (69.7)	0.803-2.423	1.395	0.237
Occupation						
Farmer	117 (56.9)	39 (33.3)	78 (66.7)	1.279-12.510	4.00	0.017*
Local trader	34 (14.8)	8 (23.5)	26 (76.5)	1.712-24.683	6.50	0.006*
Governmental employee	10 (43)	5 (50)	5 (50)	0.388-10.309	2.00	0.407
Student	17 (7.4)	11 (64.7)	6 (35.3)	3.707-73.436	16.50	0.000*
Daily labour	42 (18.3)	4 (9.5)	33 (78.6)	0.252-4.714	1.091	0.907
Other	13 (4.3)	10 (76.9)	3 (23.1)		1	

Residence						
Urban	97(42.2)	31(32)	66(68)		1	
Rural	133(57.8)	46(34.6)	87(65.4)	0.509-1.550	0.888	0.667
Contact history						
Yes	21(9.1)	10 (47.6)	11 (52.4)	0.210-1.282	0.519	0.155
No	209 (90.9)	67 (32.4)	142 (68)		1	
Chronic cough						
Yes	199(86.5)	66(32.2)	133(66.9)	1.136-7.589	2.937	0.84
No	31(13.5)	11(35.5)	20(64.5)		1	
Bacterial load						
Scanty	25 (10.9)	13 (52)	12 (48)		1	
1^+	89 (38.7)	18 (20.2)	71 (79.8)	1.670-10.936	4.273	0.002 *
2^+	76 (33)	30 (39.5)	46 (60.5)	0.669-4.125	1.661	0.274
3^+	40 (17.4)	16 (40)	24 (60)	0.593-4.452	1.625	0.345
Drug exposure						
Yes	65 (28.3)	38 (58.4)	27 (41.5)	2.470-8.370	4.547	0.000*
No	165 (71.7)	39 (23.6)	126 (76.4)		1	
Knowledge score (KAP)						
Poor	133 (57.8)	37 (27.8)	96 (72.2)	1.046-3.170	1.821	0.034*
Good	97 (42.2)	40 (58.8)	57 (58.8)		1	

a-Number in parentheses is row percentage of respondents, 1- reference category

Multivariate logistic analysis was revealed that only previous drug exposure and 1^+ bacterial load had significance association with development of drug resistance cases. Previous exposure for anti tuberculosis drugs contribute 6.4 times for the DR-TB strains (OR=6.452, 95% CI = 2.763-15.068, P-value=0.000). Similarly cases harboured 1^+ bacterial load contributed six times for the development of DR-TB bacilli (OR=6.302, 95 % CI = 2.103-18-880, P-value=0.001) (Table 5).

Table 5: Multivariate analysis of characteristics associated with development of drug resistance among smear positive cases in Eastern Amhara Region, North East Ethiopia February, 2011.

Variables	Odd Ratio	95% CI	P-value
Previous drug exposure			
Yes	6.452	2.763-15.068	0.000*
No	1		
Bacterial load			
Scanty	1		
1^{+}	6.302	2.103-18.886	0.001*
2+	1.861	0.660-5.250	0.240
3+	2.041	0.640- 6.508	0.228
Score knowledge (KAP)			
Poor	1.241	0.549-2.805	0.604
Good	1		
Occupation			
Farmer	2.537	0.863-7.460	0.091
Local trader	0.380	0.079-1.820	0.226
Daily labour	3.084	0.896-10.613	0.074
Student	0.969	0.276-3.407	0.961
Other	1		

5. 2. Knowledge, Attitude and Practice towards tuberculosis

5.2.1. Socio demographic characteristics

A total of 422 (230 smear positive and 192 smear negative) respondents were participated in this study. Of these, 221(52.4%) and 201(47.6%) were males and female respectively. Male to female ratio was 1.1: 1. The mean age of the respondents was 34 years ranging from 18 to 80 years old and half of respondents were belongs to 18-30 years age group. About 78.7 % (332) were married and 61.8 % (261) were residing on rural area. Farming was the means of livelihood for most 207(49.1%) study subjects. Similarly 41.9 % (177) were illiterate and 7.1 % (30) were able to read and write. Of the respondents majority of them 54% (228) were Amhara and 48.8 % (206) were muslim. Among the study subjects 41.2 % (174) had five or more family members (Table 6).

Table 6: Socio –demographic characteristics of smear positive and negative patients in Eastern Amhara Region, North East Ethiopia, February 2011

Variables	Number	percent (%)
Age group (year)		
18-30	212	50.2
31-40	91	21.6
41-50	79	18.7
>=51	40	9.5
Sex		
Male	221	52.4
Female	201	47.6
Religion		
Muslim	206	48.8

Christian	216	51.2
Education		
Elementary &junior	104	24.6
High school	67	15.9
College	22	5.2
Illiterate	177	41.9
Religious school	22	5.2
Literacy class	30	7.1
Residence		
Urban	161	38.2
Rural	261	61.8
Occupation		
Farmer	231	54.7
Governmental employee	23	5.5
Local trader	70	16.6
Daily labour	46	10.9
Student	37	8.8
Other	15	3.6
Ethnicity		
Amhara	228	54
Oromo	86	20.4
Tigray	17	4.0

Agew	91	21.6
Marital status		
Married	332	78.7
Unmarried	90	21.3

5.2.2. Tuberculosis awareness and sources of information

Most study subjects 281 (66.6 %) heard about PTB from health professionals, while 160 (37.9%) from person to person communication (family, friends and neighbors), 101(23.9%) from public radio, 75 (17.8%) from television, and 42 subjects (9.9%) from teachers for the first time. Among respondents only 12.3 % (52) tried to searching information about TB and 99.8 % (421) feel that they were not well informed about TB (Table 7). The three best source of information mentioned by respondents to promote KAP about TB were health workers 223(52.8%), public radio 212(50.2%) and television 210 (49.8%).

Table 7: Respondents source of information about general aspects of TB in Eastern Amhara Region, North East Ethiopia, February 2011

Variables	Frequency	Percent
Source of information		
Health workers	281	66.6
Radio	101	23.9
Teachers	75	17.8
Family, friends, neghbour	160	37.9
Teachers	42	9.9
Brochurs	97	23
Other	19	4.5

5.2.3. General Knowledge about TB

Inhaled droplets through coughing and sneezing were recognized as the common source of TB infection by 79.9% respondents, but exposure to dust (65.4 %), exposure to cold (62.6 %), drinking raw milk (44.8 %), eating together (37.2 %), sharing dish (30.1%), hand shaking (1.2%) were also mentioned as important modes of transmission.

The four most commonly recognized symptoms of TB mentioned by respondents were coughing (65.6 %), weight loss (33.2 %), cough > 2 weeks (32.7 %) and shortness of breath (29.4%). Of respondents, 281 (66%) respondents considered covering mouth and nose as the most commonly used method for preventing the spread and transmission of TB. Moreover 53.3% respondents mentioned that transmission and spread of TB could be prevented by good nutrition, closing windows (46%), prevent from sex (46.2%), and separating dish (28.9%).

Majority 76.8 % (324) believed that TB is a curable disease and 60.4% (255) of anybody were at risk of acquired the disease. Similarly 278 (65.9%) stated that the disease is curable with modern therapy but 22.5 %(95) of respondents not knew how it was cured. Only 43.1% of respondent knew current free service of diagnosis and treatment of TB and 50.5% not knew its service fee (Table 8**)**.

Table 8**: Respondents knowledge about general aspects of TB in Eastern Amhara region, North East Ethiopia, February 2011**.

Variables	**Frequency**	**Percent**
Sign/symptom		
Cough	277	65.6
Cough > 2 weeks	138	32.7
Chest pain	114	27.0
Ongoing fatigue	112	26.5
Shortness of breath	124	29.4
Weight loss	140	33.2
Fever	70	16.6
Other	21	4.9
Do not know	57	13.5
Mode of acquiring		
Through air droplet	337	79.9
Through shaking hands	5	1.2
Through sharing dish	127	30.1
Through good nutrition	157	37.2

Closing windows	189	44.8
Do not know	61	14.5
Mode of prevention		
Covering mouth &nose	281	66.6
Avoiding shaking hands	30	7.1
Avoiding sharing dish	122	28.9
Through good nutrition	225	53.3
Closing windows	194	46
Do not know	33	7.8
Person at risk		
Anybody	255	60.4
Only poor	54	12.8
Only person alcoholic	87	20.7
Only live with HIV/AIDS	126	29.9
Only alcolics	62	14.7
Other	7	1.7
Curability of TB		
Yes	324	76.8
No	3	0.7
Do not know	95	22.5
How TB cures		
Anti-tuberculosis drugs	278	65.9
Herbal remedies	15	3.6

Home rest without medicine	7	1.6
Do not know	95	22.5
Diagnosis and treatment fee		
Free of charge	182	43.1
Reasonably priced	22	5.2
Somewhat/moderately expensive	7	1.7
Do not know	213	50.5
Knowledge score		
Poor	226	53.6
Good	196	46.4
Previously treated		
Yes	76	18
No	346	82

5.2.4. Attitudes and Practices

In studying the attitudes and practices of respondents, 36.5%(153) study subjects thought PTB as very serious, those who thought as somewhat serious accounted 18.5% (78), and respondents that feel TB as not very serious diseases accounted 23.7 % (100) but 21.6% not knew the seriousness of TB both globally and nationally. About 58.3 % of respondents (246) fear if they had TB. Only 24.4% (103) respondents told presence of TB for their close friends freely and 37.4% (158) respondents thought that might be acquiring by TB disease (Table 9).

From open ended questions majority of respondents worried about the disease due to transmitted to their family, fear of stigma (social interacts), might not be cured, and unable to done work especially farmers.

About 45.3% (191) respondents pursue self treatment option as choice for primary health care; however only 13.7 % (58) respondents visited governmental clinic/hospital two or more times per year. Respondents mentioned cost 69.9 %(295) and difficulties in transportation 54.5 %(230) as the main reason for their delaines to seeking care. About 20.4 %(86) respondents were not sure where to go to found care (Table 9).

Table 9: Respondents` Attitude and Practice about TB in Eastern Amhara Region,North East Ethiopia, February 2011.

Variables	Frequency	Percent
Do you think acquired by TB		
Yes	158	37.4
No	264	62.6
Thought on seriousness		
Very serious	153	36.3
Somewhat serious	78	18.5
Not very serious	100	23.7
Reaction if TB had		
Fear	246	98.3
Surprise	23	5.5
Shame	23	5.5
Sadness or hopeless	54	12.5
Do not worry	76	18
Telling presence of TB		

Parents/family	389	92.2
Doctor	368	87.2
Close friends	103	24.4
No one	5	1.2
Choices of care		
Health facilities	65	15.4
Pharmacy	104	24.6
Traditional healer	62	14.7
Pursue self treatment option	191	45.3
Frequency of visit		
Twice or more / yr	58	13.7
Once/ yr	90	21.3
<once /yr,at least twice/5 yrs	120	28.4
Once in past 5 yrs	90	21.3
Never in past 5 yrs	64	15.2
Reason for delay		
Difficulties in transportation	230	54.5
Not sure where to go	86	20.4
Cost	295	69.9

Source of information: Public radio as a source for TB related information significantly associated with literacy, sex and residence status ($p < 0.05$) (Table, 10, 11, 12) and health workers only with sex ($p = 0.014$), (Table 11). Similarly television, teachers, personal

experience, brochures and posters significantly associated with residence status ($p < 0.05$) (Table 12).

Table 10: Dissemination of information about TB by literacy status of study subjects (per cent in Bracket) in Eastern Amhara Region, North East Ethiopia, February 2011.

Source of information	Literate (n=192)	Illiterate (n=230)	P-value
Radio	55 (28.6)	46 (20)	0.038*
Television	39 (20.3)	36 (15.7)	0.212
Friend, family , neighbours	72 (37.5)	88 (38.3)	0.646
Teachers	15 (7.8)	26 (11.3)	0.228
Health workers	119 (62)	162 (70.4)	0.067
Bill board	4 (2.1)	4 (1.7)	0.796
Brochures, posters	14 (7.3)	9 (3.9)	0.128

*- shows significance difference

Table 11: Dissemination of information about TB by sex status of study subjects (per cent in bracket) in Eastern Amhara Region, North East Ethiopia, February 2011.

Source of information	Male (n=221)	Female (n=201)	P value
Radio	64 (29)	37 (18.41)	0.011*
Television	41 (18.6)	34 (16.9)	0.660
Friends, family, neighbours	80 (36.2)	80 (39.8)	0.418
Teachers	20 (9.04)	21 (10.4)	0.628
Health workers	159 (71.9)	122 (60.7)	0.014*
Bill board	5(2.3)	3 (1.5)	0.562
Brochures, posters	14(6.3)	9 (4.5)	0.401

*- shows that significance difference occur

Table 12: Dissemination of information about TB by residence status of study subjects (percent in bracket) in Eastern Amharra Region, North East Ethiopia,February 2011.

Source of information	Urban (n=161)	Rural (261)	P value
Radio	65 (40.4)	36 (13.8)	0.000*
Television	65 (40.4)	10 (3.8)	0.000*
Health workers	107 (66.5)	174 (66.7)	0.965
Teachers	6 (3.7)	35 (13.4)	0.001*
Bill board	4 (2.5)	4 (1.5)	0.486

Family, friends, neighbours	44 (27.3)	116 (44.4)	0.001*
Brochures, posters	14 (8.7)	9 (3.4)	0.021*

Respondents` knowledge score: The overall respondents' knowledge score regarding the cause , transmissibility, signs/symptoms, modalities of prevention, person at risk, awareness of charge of treatment and diagnosis, and its curability was computed (maximum of 13 scores). The mean and median knowledge score of respondents was 6.81 and 7 respectively. Using median score as cut off, majority (53.6%) had poor knowledge score (Table 14).

Previous history of contracting TB: Respondents who contracted the disease before had good knowledge score about different clinical sign/symptom, transmission, prevention and cost of TB treatment and diagnosis than no history of contracting the disease (Table 15).

Pulmonary smear positive cases: Out of pulmonary smear positive respondents, who are highly contributing to the spreading out of the infection, only 43.5% suspected that might be acquired by TB. Of smear positive cases 38.3% (88) not knew current free cost of TB diagnosis and treatment. Moreover 14.8 %(34), 12.2 %(28), 14.3 %(33), 19.1 %(44) smear positive respondents not knew expected sign/symptom, transmission, prevention, and how TB is cure respectively.

Age : Highest rate (55.6%) of knowledge score were observed on younger age group(18-30) years and its knowledge rate decreased to 21.9%, 15.3% and 7.1% with age group 31-40, 41-50 and >=51years respectively (Table 14).

Residence: Rural residence was less aware of different aspect of TB like its sign/symptom, transmission, cause, prevention, current treatment and diagnosis modalities than urban residence (Table 14, 15).

Education: Illiterates were less aware of different aspect of TB like its sign/symptom, transmission, cause, prevention, and current treatment, diagnosis modalities than literate (Table 14, 15).

Gender: Males were more aware than female about different aspect of TB disease, for instance cough > 2 weeks (52.9% vs 47.1%), weight loss(56.4% vs 51.4%), transmitted through air

droplets (53.1% vs 46.9%) and prevented by covering mouth and nose (52.35 vs 47.7%) respectively (Table 13). Only 23.2% of the male and female (n=98) respondents had good knowledge about the disease (Table 14).

Table 13: Awareness and sources of information about general aspects of TB according to sex, literacy and residence status of respondents in Eastern Amhara Region, North East Ethiopia, February 2011.

Variables	Male	Female	Literate	Illiterate	Rural	urban
Source of information						
Health workers	159(56.6)[1]	122(43.4)[1]	119(42.3)[1]	162(57.6)[1]	174(61.9)[1]	107(38.1)[1]
Person to person	80(50)	80(50)	72(45)	88(55)	116(72.5)	44(27.5)
Radio	64(63.4)	37(36.6)	55(54.5)	46(45.4)	36(35.6)	65(64.3)
Television	41(54.7)	34(45.3)	39(52)	36(48)	60(13.3)	65(86.5)
Teachers	20(48.8)	21(51.2)	15(36.6)	26(63.4)	35(85.4)	6(14.6)
Bill boards	5(62.5)	3(37.5)	4(50)	4(50)	4(50)	4(50)
Sign/symptoms						
Productive cough	143(51.6)	134(48.4)	133(48.1)	144(52)	155(56)	122(44.0)
Cough > 2 weeks	73(52.9)	65(47.1)	69(50	69(50)	65(47.1)	73(52.9)
Chest pain	61(53.5)	53(46.5)	55(48.2)	59(51.8)	64(56.1)	50(43.9)
Shortness of breath	64(51.6)	60(48.4)	60(48.4)	64(51.6)	65(52.4)	59(47.6)
Ongoing fatigue	60(53.6)	52(46.4)	49(43.8)	43(38.4)	65(58)	47(42)
Weight loss	79(56.4)	61(43.6)	72(51.4)	68(48.6)	69(49.3)	71(50.7)
Nausea	71(58.3)	5(4.7)	3(25)	9(75)	8(66.7)	4(33.3)

Sever headache	5(55.6)	4(44.4)	3(33.3)	6(66.7)	5(55.6)	4(44.4)
Fever	35(50)	35(50)	36(51.4)	34(48.6)	44(62.8)	26(37.1)
Mode of transmission						
Cough and sneezes	179(53.1)	158(46.9)	157(46.6)	180(53.4)	192(57)	145(43)
Hand shaking	4(80)	1(20)	2(40)	3(60)	4(80)	1(20)
Sharing dish	67(52.8)	60(47.2)	60(47.2)	67(52.8)	71(55.9)	56(44)
Eating (same plate)	81(51.6)	76(48.4)	79(50.3)	78(49.7)	89(56.7)	68(43.3)
Drinking unboiled milk	94(49.7)	95(50.3)	93(49.2)	96(50.8)	114(60.3)	75(39.7)
TB diagnosis and treatment fee						
Free of charge	119(55.9)	94(44.1)	114(53.5)	99(46.5)	107(50.2)	106(49.8)
Reasonably priced	2(100)	0	0	2(100)	2(100)	0
Somewhat/moderately expensive	2(28.6)	5(71.4)	1(14.3)	6(85.7)	4(57.1)	3(42.8)
Mode of prevention						
Covering mouth &nose	147(52.3)	134(47.7)	138(49.1)	143(50.9)	156(55.5)	125(44.5)
Avoide hand shaking	13(43.3)	17(56.7)	20(66.7)	10(33.3)	22(73.3)	8(26.7)
Avoid sharing dish	65(53.3)	57(46.7)	64(52.4)	58(47.5)	69(56.6)	53(43.4)
Closing windows	96(49.5)	98(50.5)	101(52)	93(47.9)	112(57.7)	82(42.3)
Good nutrition	117(52)	108(48)	107(47.6)	118(52.4)	130(57.8)	95(42.2)
Curability of TB						
Yes	173(53.4)	151(46.6)	159(49.1)	165(50.9)	180(55.5)	144(44.4)
No	3(100)	0	1(33.3)	2(66.7)	2(66.7)	1(33.3)
How TB cure						
Anti-TB drug	148(53.2)	130(46.8)	138(49.6)	140(50.3)	151(54.3)	127(45.7)

Home rest with out medicine	2(28.6)	52(71.4)	49(57.1)	3(42.9)	7(100)	0
Herbal remedies	1(100)	0	0	1(100)	0	1(100)
Thought on seriousness						
Very serious	83(54.2)	70(45.8)	80(52.3)	73(47.7)	87(56.9)	66(43.1)
Some what serious	38(48.7)	40(51.3)	35(44.9)	43(55.1)	43(55.1)	35(44.9)
Not very serious	49(49)	51(51)	46(46)	54(54)	62(62)	38(38)

1 - Number in parentheses is row percentage of respondents

On univariate analysis illiterates (OR = 0.634, 95% CI=0.431 – 0.933, P-value = 0.021), rural residents (OR = 0.421, 95% CI= 0.281-0.628, P-value = 0.000), non-previous history of contracting TB (OR = 0.380, 95% CI = 0.226-0.638, P-value = 0.000), self treatment options experience (OR = 0.403,95% CI = 0.227 – 0.718, P-value = 0.002), delayed frequency of visit: once per year (OR = 0.492, 95%CI = 0.246 – 0.984,0.045), less than once per year but at least twice in five years (OR = O.435, 95%CI = 0.225 -0.843, P-value = 0.014), once in last five years (OR = O.300, 95%CI = 0.149 – 0.603,P-value = 0.001), never in five years (OR = 0.126, 95% CI = 0.56 -0.284, P-value = 0.000) were more likely to had a low level of knowledge score (< =7). But there was no significant difference in PTB knowledge score by respondents' age group, occupation, marital status, smear result (positive and negative), zonal distribution and sex (p >0.05), (Table 14).

Table 14: Univariate analysis of respondents' characteristics related with low PTB knowledge score in Eastern Amhara region, North East Ethiopia, February 2011.

Variables	total number	poor score[1]	good score[2]	95% CI	OR	P-value
Residence						
Rural	261(61.8)[3]	161(61.7)[3]	100(38.3)[3]	0.281-0.628	0.421	0.000*
Urban	161(38.2)	65(40.4)	96(59.6)		1[4]	
Education						
Literate	192(45.5)	91(47.4)	101(52.6)		1	
Illiterate	230(54.5)	135(58.7)	95(41.3)	0.431-0.933	0.684	0.021*
Choice of care						
Governmental facilities	65(15.4)	26(40)	39(60)		1	
Pharmacy	104(24.6)	49(47.1)	58(55.8)	0.422-1.475	0.789	0.458
Traditional healer	62(14.7)	32(51.6)	27(43.5)	0.276-1.148	0.563	0.114
Pursue self treatment option	191(45.3)	119(62)	72(37.5)	0.227-0.718	0.403	0.002*
Frequency of visit						
Twice or more/yr	58(13.7)	18 (31.0)	40 (69)		1	
Once /yr	90(21.3)	43(47.8)	47(52.2)	0.246-0.984	0.492	0.045*
<once/yr,at least twice in 5 yrs	120(28.4)	61(50.8)	59(49.2)	0.225-0.843	0.435	0.014*
Once in past yrs	90(21.3)	54(60)	36(40)	0.149-0.603	0.300	0.001*
Never in 5 yr	64(15.2)	50(78.1)	14(21.9)	0.560-0.284	0.126	0.000*
Gender						
Male	221(52.4)	123(55.6)	98(44.3)		1	

Female	201(47.6)	103(51.2)	98(48.8)	0.814-1.752	1.194	0.364
Age group						
18-30	212(50.2)	103(48.6)	109(51.4)		1	
31-40	91(21.6)	48(52.7)	43(47.3)	0.518-1.384	0.847	0.507
41-50	99(18.7)	49(62)	30(38)	0.341-0.981	0.579	0.142
>=51	40(9.5)	26(65)	14(35)	0.252-1.028	0.509	0.060
Previous treatment with TB						
Yes	76(18)	26(34.2)	50(65.8)		1	
No	346(82)	200(57.8)	146(42.2)	0.226-0.638	0.380	0.000*
Occupation						
Farmer	231(54.7)	154 (66.7)	77(33.3)	0.330-3.028	1.000	1.000
Governmental employee	23(5.5)	3(13.6)	20(87)	0.874-9.148	2.828	0.083
Local trader	70(16.6)	29(41.4)	41(58.6)	2.63-67.38	13.333	0.072
Daily labour	46(10.9)	20(43.5)	26(56.5)	0.766- 8.821	2.600	0.125
Student	37(8.8)	10(27)	27(73)	1.478- 19.700	5.400	0.061
Other	15(3.6)	10(66.7)	5(33.3)		1	
Marital status						
Married	332(78.7)	184(55.4)	148(44.6)	0.441-1.123	0.704	0.141
Unmarried	90(21.3)	42(46.7)	8(53.3)		1	

1 –knowledge score < =7.00 (maximum 13 score when 7.00 was used as cut off for comparison), 2-knowledge score > 7.00, 3-number in parentheses are row percentage of respondents, 4- reference category, *- indicates that those variables that had significance associations with low knowledge score ($p < 0.05$)

Multivariate logistic analysis was conducted on independent variables that significantly associated ($p < .05$) with low knowledge score and the findings revealed that illiterates, non-

previous history of contracting TB, self treatment option experience, delayed frequency of visit: once in past five years and never in past five years, and rural residence were independently associated with low knowledge score with inversely relation (Table 11**).**

Table 15: Multivariate analysis of characteristics associated with low knowledge score among smear positive and negative patients in Eastern Amhara Region, Ethiopia February 2011.

Variables	Odd ratio	95% CI	P- value
Residence			
Rural	0.440	0.292-0.662	0.000*
Urban	1		
Education			
Illiterate	0.694	0.465-0.949	0.021*
Literate	1		
Choice of care			
Governmental health facilities	1		
Pharmacy	1.121	0.569-2.208	0.741
Traditional healer	0.785	0.362-1.701	0.540
Pursue self treatment option	0.458	0.253-0.828	0.010*
Frequency of visit			
Twice or more /yr	1		
Once a year	0.610	0.292-1.278	0.190
< once /yr ,at least twice /5 yrs	0.514	0.253-1.044	0.660
Once in past 5 yrs	0.427	0.202-0.904	0.026*
Never in past 5 yrs	0.154	0.065-0.368	0.000*

Previous treatment with TB			
Yes	1		
No	0.556	0.312-0.993	0.047

*- indicates variables independently influence occurrence of low knowledge score.

6. Discussions

6.1. Drug Susceptibility Testing

In the present study the overall drug susceptible rate for four (HRSE) first line drugs was found in 66.5% (Table 2). This susceptible rate was lower than in the previous studies done in Ethiopia (34-35, 37) and this indicates that timely emergence of those resistance strains. Similarly the overall drug resistance rate for one or more drugs was found in 33.5% (Table 2). A comparable level of drug resistance for one or more drugs reported in Saudi (29.7%), in South Africa (30.2%) and in Ethiopia (29.4%) (28, 30, 37). This finding was higher than in the previous studies done in Ethiopia (15.7 - 23.7 %), in Turkey (21.1%), in Iran (14.1%) (26, 29, 34-35) but lower than study done in Ethiopia (50-53.2%), in Taiwan (55.5%), in Pakistan (64%) and in Iran (41.6%) (23, 25-27, 29, 33-36). In this study 6.5% of resistance cases was MDR-TB (Table 2), which was higher than those previously reported in Iran (2.8%) but lower than reported in Saudi (20%), and in Pakistan (47%) (25, 27-28). Similarly this study depicted that mono resistance found in 17.4 % (40) (Table 2). A comparable level of single drug resistance reported in Nigeria (15%), in Kuwait (15.3%) and in Taiwan (15.9%) but lower level of single drug resistance reported in Pakistan (7.9%) (22-24, 31). This may reflect the variations in the studied population and variation on load of drug resistance strains in different geographical location.

The resistance rate observed for Isoniazid in this study was 17.7% (41) (Table 2). In line with this previous studies in Ethiopia showed that the frequency of resistance to H was within a range of 2%-21% (10). But this finding was lower than reported in Saudi (28.7%) (28). Single drug resistance rate for H in this study was 5.2% (Table 2). A study conducted in Taiwan showed a similar rate of resistance (5.1%) (22). While it was lower than previous study in Ethiopia (13.3%) and in Kuwait (8.4%) (22-24, 31). This may be due to difference in method used for DST analysis and study subject variation.

The resistance rate to Streptomycin in this study was (27.0%) (Table 2) and a comparable level result were reported by Kassue *et al.* (24.3%) in Ethiopia (37). This findings has increased when compared with previous studies done in Ethiopia (2 to 20%) and in Saudi (22.8%) (13, 28). This may be indicate that previous utilization of S as mono therapy for TB on DOTS service, poor

controlling condition that led to multiplication of resistance strains including poor adherence of patients, provision of poor drug quality and regimens, administration of incorrect drug combination and utilization for treating other bacterial infection. Single drug resistance rate to S in this study (13.3%) has increased when compared with previous studies done in Taiwan (0.7%), in Kuwait (5.2%) but lower than report in Ethiopia (16.25%) (Table 2) (23-24, 34). This lower finding in this study might be explained due to method variation and study participants were known HIV sero-positive cases and result in higher drug resistance.

The resistance rate to Rifampicin in this study (10.0%) (Table 2) has increased when compared with previous studies done in Ethiopia (1.8%) but lower than in Saudi (20.8%) (28, 35, 37). Rifampicin resistance need attention since this drug is core components of the standard DOTS of TB. Moreover single drug resistance rate for R was 0.86 %(Table 2); lower level of resistance reported in Taiwan (0.5%) but higher level of mono drug resistance reported in Kuwait (1.7%) and in Ethiopia (1.2%) (23-24, 34). This indicates that R mono resistance is low and correlates with resistance of other drug.

Similarly resistance rate to Ethambutol in this study was 6.5% (Table 2). A study conducted in Saudi showed a comparable rate of resistance (6.9%) (28). But this report was higher than previous study reported for over all resistance for E in Ethiopia (35). Single drug resistance for E (nill) in our findings was lower than previously reported in Taiwan (1.6%), in Kuwait (1.5%) and in Ethiopia (3.5%) (Table 2) (23-24, 34). This lower finding in Ethiopia may be advantageous that should be exploited in order to develop a regimen for the management of MDR-TB.

In the present study majority of single drug resistance cases were occurred by S followed by H (Table 2). Demissie *et al*. in Ethiopia revealed that majority of single drug resistance was occurred by S followed by H (35). However there were different report regarding the frequency of different first line drug like Khan *et al.* in Saudi described that resistance was most common to H followed by S and R respectively (28) and Suwei *et al*. in Taiwan showed that majority of single drug resistance occurred by H followed by R (23). This variation may be due to utilization of different first line drugs as mono therapy, difference attention for controlling system and geographical variation in load of resistance cases.

In this study drug resistance rate for two drugs were 8.7% and majority of cases occurred by HS followed by SR. Similarly drug resistance rate for three drugs were 3.04% and majority of this cases occurred by HRS (Table 2). Aamer *et al*. in Pakistan depicted that majority of two drugs resistance cases occurred by HR, HS and SR respectively and majority of three drug resistance cases occurred by HRS (22). The dominance of combination of two drugs HR rather than HS in this finding might be due to high load of MDR-TB in Pakistan and surveillance was nation wide.

Many study showed that drug-resistant TB had a statistically significant association with exposure of anti-tuberculosis drugs (25, 27, 29-30, 32). In consistent with this, our finding also observed that the odds of favoring drug resistance in previously treated case were 6.4 times more than new cases (Table 5).

In the present study drug resistance for one or more drugs observed in new cases was 23.6%. This finding was higher than those reported in Kuwait (10.3-15.3%) and in Ethiopia (15.6%) but lower than in Myanmar (29.3%) and in Pakistan (39%) (24-25, 32, 34). Inaddition this study revealed that 1.8% MDR-TB cases were observed in new cases (Table 3). In line with this WHO 2010 reported that MDR-TB range within 0 to 28.3% in new cases (21). This finding was higher than in previous study done in Ethiopia (nill to 1.2%) but lower than study done in Turkey (4.4%), in Nigeria (4%), in Myanmar (4.2%), and in Pakistan (10%) (13, 25, 29, 31-32, 37). The high rates of resistance among new cases in this study may indicate that either drug resistant strain is circulating and transmitted in the community or presence of inappropriate control and prevention system including delay in diagnosis that favour transmission of resistance strains. Transmission of those resistant strains is a serious problem and threat, as it is difficult to treat patients infected with drug resistance strains.

Similarly in the present study drug resistance for one or more drugs observed in previously treated case was (58.5%) (Table 3), which was higher than those reported previously in Ethiopia (53.6%), and in Myanmar (45%) but lower than reported in Pakistan (79%) (25, 32-33). Moreover 18.46% MDR-TB cases were observed in previously treated cases (Table 3). A comparable level of MDR-TB observed in Myanmar (18.4%) and in Nigeria (18%) (31-32). WHO 2010 also showed that MDR-TB range from 0% to 48% in previously treated cases (21). Higher level of MDR-TB reported in Turkey (22.4%), in South Africa (58.4%), in Ethiopia

(26.2%) and in Pakistan (79%) (25, 29-30, 33). High load of resistance among re-treatment cases indicated that resistant mutants naturally occurring in the Mycobacterial population are selected and multiply by inadequate or interrupted treatment with anti-tuberculosis agents. Failure to adhere TB control and prevention program may furthers aggravate the risk of development of drug resistance strains.

In this study 13.33% and 27.69% of the strains isolated from new and previously treated patients were resistance to one drug respectively. Similarly 7.8% vs 10.7% for two drugs, 1.8% vs 6.2% for three drugs and 0.6% vs 13.8% for all four first line drugs on new and previously treated cases respectively (Table 3). This indicated that resistance strains both in new and previously treated cases were emerged especially for H and S. Emergence of those resistance strains especially for the most bactericidal anti-tuberculosis agent H may further aggravated the emergence of MDR-TB and it may threat TB control program.

In the present study eventhough higher number of younger age group and males harbor drug resistance strains, both age group and sex (Table 4) had not any significant association with harbouring of drug resistance (Table 4). But Shamaei *et al*. in Iran, Suwei *et al*. in Taiwan and Green *et al*. in South Africa showed that DR-TB significantly associated with age group (23, 27, 30). However WHO 2010 reported that due to conflict data harboring of drug resistance strains might not associated with age group rather the association of resistance strains with either on younger or older group may reflects TB epidemic is recent or past years (1). Moreover Surucuoglu *et al*. in Turkey reported that more males than females significantly harbour resistance drug strain (29). However WHO 2010 report showed that, eventhough males predominantly harbouring resistance strain of TB, the overall risk of harbouring drug resistance strains are not influenced by sex rather may be due to differences in access to health-care services or exposure to other risk factors (21). Present finding also consistent with this report. Moreover presence of cavitary condition and high bacillary load in the lung contribute for emergence of drug resistance strain (9), in the present study bacterial load (1^{+}) independently contributed 6.3 times for the development of drug resistance strains (Table 5). It may be due to poor socio-economic status and it needs further large scale investigation.

6.2. Knowledge, Attitude and Practice

A questionnaire based survey was conducted among new and follow up patients referred from OPD and DOTS for AFB examination on selected health facilities of Eastern Amhara region.

This study demonstrated that majority (53.6%) of respondents had low knowledge score (Table 14). This finding was in agreement with others (38, 41, 45-46, 49). Similarly several study explained that majority of respondents heard about TB from health workers, television, and personal experience (42, 44-46, 49) for the first time. Mengistu *et al*. in Ethiopia depicted that health workers, friends/relatives and radio as main source of information (49). This study also showed that 66.6%, 37.9%, and 23.9 % of respondents heard about PTB for the first time from health professional, personal experience and radio respectively (Table 7). This indicate that health workers, radio, and personal experience were act as successful means of disseminating information about TB and it were promising venues for better detection of TB cases. However lowered finding of television (17.8%) as sources of information in present study might be due to majority of study subjects were rural residence and those were inaccessible to television (Table 6). This also shows that the importance of integrating all the available measures for reaching to people for dissemination of information. This study revealed that sources also differed among sex (Table 11). This was in agreement with study conducted by Hoa NP *et al*. in Vietnams (42)

Moreover only 12.3 % of respondents tried to searching information about TB and 99.8 % (421) feel that they were not well informed about TB. This reflects that requirements of implementing ACSM program to raise awareness of patients about TB.

Based on present finding majority (79.9%) of the respondents mentioned that TB is transmitted by respiratory droplets through coughing and sneezing, and prevented by covering mouth and nose (66%) (Table 8). This was in agreement with study done in Ethiopia and Pakistan (44, 49). The present study also observed that there were numerous misconceived ideas about its cause, transmission and prevention that needs clarification like exposure to cold, dust, drinking raw milk, closing windows, eating together, prevent from sex and sharing dish (Table 8). Those misconceived ideas might have a potential to create ground for stigmatization of TB patients. Study conducted in Pakistan, Croatia and Ethiopia also showed that there were several

misconceptions regarding TB that need to be clarified (44, 48-49). Moreover in the present study eventhough cough (65.6%), weight loss (33.2%), cough > 2 weeks (32.7%) explained as the three main symptoms of TB (Table 8), majority of respondents were unaware of different symptoms of TB. This finding was in consistent with a study conducted by Hoa *et al*. and Mushtaq *et al.* and result revealed that a large proportion respondents' knowledge regarding TB was deficient in all aspects (43, 46).

About 76.8% respondents also explained TB as a curable disease and 65.9 % (278) curable with modern therapy and 60.4 % of anybody were at risk of acquired the disease. But only half of respondents knew the free charge of TB diagnosis and treatment (Table 8). This was in agreement with study conducted by Mushtaq *et al*. in Pakistan (45-46).

Present study revealed that majority (58.3%) of respondents feared and worried if TB had and only 24.4% respondents told presence of TB for their close friends freely (Table 9). Majority explained that fear, worried, and closure about present of TB disease result from fear of stigma, and unable to do work. This finding was concordant with other studies conducted in Iraq, Tanzania and Norway (38-39, 47). This finding also indicate the need to strengthen health education activities like IEC about TB and KAP towards the seriousness of the disease, cause and the modes of transmission, the sequelae of treatment interruption and the curability of TB.

Several studies also indicated that educational background (43, 45, 47-49), residence (46-47, 49) and age group (48) of respondents was important determinants of TB knowledge. Similarly more knowledge level was also observed among males than females (40-42). The present study found that low level of knowledge score significantly associated with being illiterate and rural residence (Table 14, 15). Sex and age group wise knowledge difference not observed in this study (Table 14); might be due to study subject variation and small number of sample size in contrast to other study. Moreover good knowledge score also observed among previously contracting TB disease than non contracting TB disease significantly (Table 15). This is in agreement with study conducted by Yimer *et al*. in Amhara region (51).

Health care seeking is a dynamic process that is influenced by socio-demographic, cultural and other factors (48,51). A systematic review in Norway indicated that many factors were

significantly related with seeking health care and contribute for delaines for seeking care like rural residence, low awareness of TB, self-treatment, stigma, low educational level, old age; poverty, female sex, low access (geographical or socio-psychological barriers),and traditional healer (48).

Long *et al*. in China showed that low awareness, poor knowledge; low financial capacity to pay for care and diagnostic were factors contribute for delayed health seeking (43). Irani *et al*. in Iraq and Thamer *et al*. in Tanzania showed that discrimination against TB patients by relatives and friends and a strong community attitude regarding TB as a stigma and poor knowledge were likely hinder positive health seeking behavior (38-39). Meaza *et al*. in Addis Ababa showed that the time for diagnosis in TB patients was long and appears to be associated with patient inadequate knowledge and distance to the health facilities (50). In present study also found that 69.9 % and 54.5% seeking care delaines were due to cost and difficulties of transportation respectively (Table 9). In this study delayed seeking also associated with low knowledge score, experience self treatment option, and delayed frequency of visit (Table 15). There were also indicators for presence of strong respondents` attitude towards TB and patients with TB as stigma (Table 9).

In the present study delayed health seeking practice was not associated with age, Literacy status, residence and sex (Table 14). Many investigators showed that seeking care is a result of multi-factorial process (38-39, 48, 51), not only socio-demographic but also other factors like culture, low financial capacity, health perception, stigma and socio-psychological barriers (believes) influence this behavior(38-39, 47-48,51). As a result correct knowledge and positive perception of the patient towards TB and its management is a prerequisite for them to seek early health care. This might be accelerated by proper implementation of ACSM program for TB.

7. Limitation of the study

- In this study only 230 sputum samples run for DST and associated with patients' knowledge level, but it is preferable to run all 422 sputum samples for DST and associated with knowledge level for better finding.
- HIV status of respondents was not known which hinder us to characterize the result further in HIV status.
- Only confirmed smear positive and negative pulmonary tuberculosis cases were included in the study.

8. Conclusions and recommendations

8.1. Conclusions

Of all isolates, 66.5% of was sensitive and 4.4% resistance to four first line anti-tuberculosis drugs (SHRE) while the remaining 33.5% was resistance to at least for single drug. MDR-TB was observed in 6.5% isolates. Overall resistance to S was found in 27 % (62), to R in 10 % (23), to H in 17.8 % (41), and to E in 6.5 % (15). Mono resistance found in 17.4 % (40) of all isolates. Similarly 9.6 % (22) of isolates was poly resistance. Of all isolates, 8.7% and 3.04% was resistance to two drugs and three drugs respectively.

Among new cases primary drug resistance for one or more drugs was observed in (23.6 %) and MDR-TB found in 3 (1.81%) cases, while in previously treated cases resistance to any drug found in (58.5 %) and MDR-TB in (18.46 %). Highest proportion of MDR-TB was found in treatment failure cases.

High rates of drug resistance in new and previously treated cases occurred by H and S. Drug resistance for the main anti-tuberculosis including Isoniazid may future aggravate development of MDR-TB. DR- TB isolates including MDR are emerging problem both in new and re-treatment patients. Previous exposure of drug and bacterial load were important determinant of development of drug resistance.

Moreover majority (53.6%) of respondents had limited knowledge in all aspects of most infectious form of TB. Only half of respondents were aware of current treatment and diagnosis free cost of TB. Only 36.5% of respondents knew about very serious nature of PTB and majority of TB cases were worried about the disease due to might not cured, fear of stigma, unable to do work and might be transmitted to their families. About 45.3% (191) of respondents pursue self treatment option as choice for primary health care but only 13.7% visited governmental clinic/hospital two or more times per year. Cost and difficulties in transportation were mentioned as the main reason for their delaines to seeking care.

Low knowledge score was more evident among the rural people, illiterate, non previous contracting TB, and experience self treatment options. Low knowledge score also associated

with delayed health seeking behavior. There were several misconceived ideas about cause, transmission and prevention of TB that contributes for stigmatization of TB patients and delayed health seeking behavior. Lack of awareness of PTB disease might be aggravating the risk of transmission among the patients. Therefore appropriate control measure like establishing proper information, education and communication pathway that indicate the level of severity and create proper awareness about its cause, transmission, prevention and availability of public service are very essential.

8.2. Recommendations

Based on our results the following recommendations are forwarded:

- DR-TB strains particularly MDR were emerged both in new and retreatment cases, so it is essential to address the problems of DR-TB through establishing good TB control program that encompass timely diagnosis, expansion and improving of DOTS and DOTS plus service to prevent further emergence of MDR-TB.
- High load of resistance among re-treatment cases indicated that resistant mutants naturally occurring in the Mycobacterial population are selected and multiply by inadequate or interrupted treatment with anti-tuberculosis agents. Therefore strengthening basic TB programs like good patient adherence, administration of good quality and dose combination of anti-tuberculosis drug and improving infection control measures is crucial for preventing the transmission and the selective pressure of resistant strains.
- It is recommended that if the survey would be include HIV status to assess the association of HIV status with drug resistance epidemic and sensitivity testing for second line anti-tubercle drugs.
- Hence majority of respondents had poor knowledge about TB, it would be better to strengthening health education and ACSM program for TB through health workers and mass media to increase chance for better case detection, to ensure adherence to anti-TB treatment, to improve KAP level and misconception about TB.
- It would be better to nominate animators on communities that facilitate transferring of suscepected TB case to health facilities and coordinates social mobilization activities to improve KAP of patients and prevent transmission of TB in the community.
- It is highly recommended that this survey be extended to include community levels that have a better picture of KAP of our population about TB.
- Assessment of health care providers` KAP towards TB is also recommended.

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Annex I-Questionnaires

Part 1. Patient Identification and demographic questions Date___/___/_____

101.code No_____.102. Card No. ____

103. Hospital/ health centres No.____. 104. Address: wereda___ Keble __ Tel ___

105. Age__/__/____106. Sex 1.M 2. F

107. Occupation? 1. Farmer 2. Merchant 3.governmental worker 4. Other___ (specify).

108. Living Area: 1.Urban 2. Rural

109. Religious 1. Muslim 2. Christian 3. Other _____(specify)

110. Educational level?

1. Illiterate 2. Elementary **3.** High school **4.** College 5. Religious schooling only 6. Literacy classes only

111**.** How far do you live from the nearest health clinic or hospital?

1 . 0–10 kilometers 2. 11–20 kilometers 3. 21–30 kilometers 4. More than 30 kilometers

112**.** Number of family _____.

113. Weight_____ (kg)

114. Height ____ (meter/centimetre)

115. Ethnic group____1. Amhara 2. Oromo 3. Tigray 4. Agew 5. Other _____ (specify).

Part 2. Clinical data

201. Symptom of TB,

1. Chronic cough (≥two weeks)
2. Production of sputum
3. Night sweats
4. Fatigue/tiredness
5. Shortness of breath
6. Un explained weight loss
7. Fever
8. Don't know
9. Other _____(specify)

202. Previously exposure of anti-tuberculosis drugs:

A. 1. If yes, 1. Complete 2. Default 3. Re-treatment 4. Relapse 2. No

203. 17.Administer drug regimen, fill by interviewer only

1. New _____
2. Follow up _____
3. Re-treatment _____
4. Default_____
5. Relapse _____

204. Did you miss taking your TB pill properly _____? 1. Yes 2. No, if No why? _____ (Specify)

Part 3. Contact history

301. Contact History with suspected Tb Patients. 1. If yes 1.Family 2. Friends

3. Negioubrs 4.Other _____(specify) 2. No

302**.** Is there any one in your family infected with M.tuberculosis? 1. If yes 1.Wife 2. Husbands 3.Children 4. Father 5. Mother 6. Grandfather and mother 2.No

Part 4. TB knowledge and awareness

401. Where did you first learn about tuberculosis? You can give more than one answers

1. Newspapers
2. Radio
3. TV
4. Billboards
5. Brochures, posters
6. Health workers
7. Family, friends, and neighbors
8. Religious leaders
9. Teachers
10. Other ___ (specify).

402**.** What are the signs and symptoms of TB?

1. Cough
2. Cough that lasts longer than 2 weeks
3. Chest pain
4. Shortness of breath
5. Ongoing fatigue
6. Weight loss
7. Nausea
8. Severe headache
9. Fever
10. Do not know
11. Other: ___ (specify)

403. How can a person get TB?

1. Through the air when a person with TB coughs or sneezes
2. Through handshakes
3. Through sharing dishes
4. Through eating from the same plate
5. Drinking unboiled milk
6. Other ___ (specify).

404. How can a person prevent getting TB?

1. Covering mouth and nose when coughing or sneezing
2. Avoid shaking hands
3. Avoid sharing dishes
4. Closing windows at home
5. By praying
6. Through good nutrition
7. Do not know
8. Other ____ (specify).

405. How expensive do you think TB diagnosis and treatment is in this country**?**

1. It is free of charge
2. It is reasonably priced
3. It is somewhat/moderately expensive

406. Are you taking TB medicines?

1. Yes 2. No 3. Never started

407. How often are you supposed to be taking your medicines? When? ___

1. Daily
2. Once weekly
3. Twice weekly
4. Three times weekly
5. Other: _____(specify)

408. When did you stop taking anti-tuberculosis drugs? (_____) Months ago

409. In your opinion, who can be infected with TB?

1. Anybody
2. Only poor people
3. Only homeless people
4. Only alcoholics
5. Only drug users
6. Only people living with HIV/AIDS
7. Only people in prison
8. Other ___(specify)

410. Can TB be cured?

1. Yes 2. No

411. How can someone with TB be cured?

1. Herbal remedies
2. Home rest without medicine
3. Praying
4. Anti-tuberculosis drugs given by health institute
5. Do not know
6. Other:_____(specify)

Part 5: TB attitudes

501. Do you think you can get TB?

1. Yes, because_____
2. No , because_____

502. In your opinion, how serious a disease is TB?

1. Very serious 2. Somewhat serious 3. Not very serious

503. How serious a problem do you think TB is in your country/region?

1. Very serious 3. Somewhat serious 4. Not very serious

504. What would be your reaction if you were found out that you have TB?

1. Fear
2. Surprise
3. Shame
4. Sadness or hopelessness
5. Other:____(specify)

505. Who would you talk to about your illness if you had TB?

1. Doctor or other medical worker
2. Spouse
3. Parent
4. Close friend
5. No one
6. Other:_____(specify)

506. What would you do if you thought you had symptoms of TB?

1. Go to health facility
2. Go to pharmacy
3. Got to traditional healer
4. Pursue self-treatment options (herbs)
5. Other: ____ (specify)

507. What worries you the most when you think about TB? _____

Part 6. Health-seeking behavior

601**.** Where do you usually go if you are sick?

1. Private clinic
2. Government clinic or hospital
3. Traditional or homeopathic healer
4. Clinic run by a nongovernmental organization or church
5. Other: _____(specify)

602. How often do you generally seek health care at a clinic or hospital?

1. Twice a year or more
2. Once per year
3. Less than once a year but at least twice in past 5 years
4. Once in past 5 years
5. Never in past 5 years
6. Other: _____(specify)

603. If you would not go to the health facility, what is the reason?

1. Not sure where to go
2. Cost
3. Difficulties transportation/distance to clinic
4. Do not trust medical workers
5. Do not like attitude of medical workers

Part 7: TB awareness and sources of information

701**.** Do you feel well informed about TB?

1**.** Yes 2. No

702. Do you wish you could get more information about TB?

1**.** Yes 2. No

703. What are the sources of information that you think can most effectively reach people like you with information on TB? (Please give the three most effective sources.)

1. Newspapers
2. Radio
3. TV
4. Billboards
5. Brochures, posters
6. Health workers
7. Family, friends, and neighbors
8. Religious leaders
9. Teachers
10. Other : ____(specify)

Thank you very much for participating in our study.

Part 8: laboratory information

801. Laboratory request form

Name of Health Center/Hospital _____ Date ___/____/_____

Patient's register number_____

Source of specimen: Pulmonary_____

Reason for examination: Diagnosis_____

Follow-up of chemotherapy_____

Specimen identification number _____ Date ____/__/___/_____

803. **CULTURE RESULTS: FINAL REPORT**

Laboratory serial number _____ Date specimen received _____

804. Microscopy results

Staining method: Ziehl-Neelsen ☐

Negative ☐ 1+ ☐

Not done ☐ 2+ ☐

☐

1-9 AFB ☐ 3+

805. Culture results

Culture method _____

No growth ☐ 1+ ☐ 2+ ☐

Contaminated ☐ 3+ ☐

Not done ☐ 4+ ☐

1-19 colonies ☐

806. Culture identification

Growth rate_____ Colony morphology _____

Niacin production: positive ☐ negative ☐

Nitrate production: positive ☐ negative ☐

Catalase _____ positive ☐ negative ☐

Culture identified as: Mycobacterium tuberculosis ☐

: MOTT ☐

Date _____ Signature _____

807. **Drug susceptibility result on LJ medium**

Date collected	Specimen	Laboratory serial No.	S	H	R	E
	1					
	2					

R: Resistant; S: Susceptible; C: Contaminated; Nd : Not done

Date _____ Examined by (name and signature)

Annex II - Information sheet read to the respondents

My name is Ahmed Esmael, a student of Addis Ababa University, Faculty of Medicine, School of Medical Laboratory Science. The aim of the study is to determine the pattern of drug susceptibility of *M. tuberculosis* against four first line anti-tuberculosis drugs stated by Ministry of Health on new, and re-treatment patients and patient`s KAP towards TB who attending on selected health facilities in Eastern Amhara Region.

The laboratory analysis will be conducted in EHNRI, Addis Ababa, Ethiopia. The study will be conducted through analysis of sputum sample that are positive for AFB by Ziehl Nelseon methods. Similarly those respondents who are smear negative also participated. 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 do not have any influence for your service that you want to use .In addition in your participation in the study do not have any invasive procedure, only give three consecutive sputum samples as recommended by the health personnel and each questionnaire only take 10-15 minutes. At the end of the study the results of patients who develop drug resistance strains particularly MDR-TB will be announced for each patients by their health institute and will be treated by announce to concerned body. 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.

Annex-III – Consent form prepared for study participants

I_____ here by giving my consent for giving three consecutive sputum samples as recommended by health personnel for DST analysis and to answer those KAP and DST questions. I understand there is no serious invasive procedure at the beginning as well as at the end of the study. I understand this study will be used not only for me but also for other TB positive patients. I know anti-tuberculosis drugs are available at those health facilities. I believe that at the end of study the result also explain for concerned body only for the purpose of the study.

Signature _____ Date_____

Thank you in helping with this important study

N.B: If you want to request additional information about the study, you will call by those phone numbers Contact address of PI, 0913681399, IRB address: 251-115- 538734

Annex IV - Amharic version of Questionnaires

¡õM ›”É:- ¾ "T>ዎች ›ÖnLÃ S[Í SKÁ

k” ---------- /-------/--------

101. SKÁ lØ` --------- 102 ካርድ ቁጥር _____ 103. ¾Ö?“ É`Ï~ SKÁ lØ` -----

104. ›É^h -------- ¨[Ç ------kuK? ---- eM¡ ----- 105. ÉT@------ 106. í ነ ¨”É

2. c?ƒ

107. e^ ነ. ገበሬ 2. ነጋዴ 3. የመንግስት ሰራተኛ 4. ሌላ ካለ _____

108. Sኖ]Á x ነ. Ÿ}T 2. ÑÖ`

109. GÃT*ƒ ነ. ማኅሊም 2. ክርስቲያን 3. ሌላ ካለ _____

110. የትምህርት ደረጃ 1. ያልተማረ 2. የመጀመሪያ ደረጃ 3. ሁለተኛ ደረጃ 4 ኮሌጅ

5. ሀይማኖታዊ ት/ቤት 6. ማንበብና መጻፍ ብቻ

111. በአቅራቢያዎ ያለው የጠፍ ድርጅት ከቤትዎ ምን ያህል ይርቃል? ነ. Ÿ 0-10Ÿ=T@ 2. Ÿ 11-20

Ÿ=T@ 3. Ÿ 21-30Ÿ=T@ 4. Ÿ 30 Ÿ=T@ uላÃ

112. የቤተሰብ ብዛት ---------

113. ¡wÅƒ-------- (ኪ.ግ)

114. lSƒ------ (ሜ/ሴ. ሜ)

115. wN?` ነ›T^ 2. *aV 3. ƒÓ_ 4. አገው 5. K?KA‹ _____

¡õK G<Kƒ:- ¡K=’>"M S[Í

201. የበሽታው ምልክቶች

1. የቆየ ሳል 1. አዎ 2. የለም

2. አክታ አለዎት 1. አዎ 2. የለም

3. ሚታ ሚታ ያልበዎታል 1. አዎ 2. የለም

4. ድካም ይሰሞዎታል 1. አዎ 2. የለም

5. የመተንፈስ ችግር አለዎት 1. አዎ 2. የለም

6. ባልታወቀ ምክንያት የሰውነት ክብደት መቀነስ ገጥሞዎታል 1. አዎ 2. የለም

7. መቅት 1. አዎ 2. የለም

8. አላወቀውም 9. ሌላ ካለ _____

202. Ÿ²=l uòƒ ¾+u= ug SÉH'>ƒ }ÖpSው ያውቃሉ? ነ. ›-፣ ከሆነ ነ. Ú'ነhKG<

2. ›s'ÝKG< 3. uQ¡U“ ¨pƒ SÉG'>~” ›eÅÓS¨<—M 4. ŸÚ[eŸ< u%EL ¾+u= ug ÅÓT> ¡T>ÁKG< 2. ¾KU

203. ¾T> ²²¨< SÉG'>ƒ፣ ቃለ መጠይቁን በሚያካሂደው ባለሙያ የሚሞላ

1. KËT] ----------

2. KT>Ÿ }M --------

3. KQ¡U“ LÃ KÅÑS ----------

4. K›s[Ö ----------

5. ŸÚ[c u%EL +u= KÁ²¨< ---------

204. የፀረ-ቲቢ መድሀኒቶችን ሳያቋርጡ በትክክል ይወስዳሉን? 1. አዎ 2.አልወስድም ፣ ከሆነ ለምን ____.

¡õM feƒ:- ¾}ÒLß'ƒ G<'@

301. የቲቢ በሽታ ይዟቸዋል ተብሎ ከሚጠረጠሩ ሰዎች ጋር ግንኙነት ነበረዎት? 1. ›- ከሆነ

1. u?}cw **2.** ÕÅ— **3.** ÑA[u?ƒ 4. K?KA‹_____ 2. ¾KU

302. ከቤተሰቦችዎ መካከል በቲቢ በሽታ የታመመ አለን? 1. ›- ከሆነ 1.T>eƒ 2. vM 3. ›vƒ

4. "ƒ 5 ›Áƒ (ሴትና ወንድ) 6. K?KA‹ _____2. የለም

¡õM ›^ƒ:- eK +u= ¨<kƒ" Ó"³u? SÖÃp

401. እርስዎ ለመጀመሪያ ጊዜ ስለ ቲቢ በሽታ የተሙት ከየት ነው? (ከአንድ በላይ ሚልስ መክጠት

ይችላሉ) 1. ŸÒ²?x 2. Ÿሬ=¿ 3. ŸቴK?y=¶" 4.ŸTe ¨mÁ cK?Ç 5. ከበራሪ êOፎች e°L©

SÓKÝ "K? KA‹ ¾T> }S< S[Í-‹ 6. ŸÖ?" c^}™‹ 7. Ÿu?}cw'ÕÅ—'Ñ<[u?ƒ

8. ŸGÃT• ©S] -‹ 9. ŸSUI^" 10. ŸK?KA‹ _____.

402. ¾+u= ug UM ¡„‹ U" U" "†¨<? 1. ሳል 2. ከሁለት ሳምንት በላይ የቆየ ሳል

3. ደረት ህመም 4. የመተንፈስ ችግር 5. ቀጣይነት ያለው ድካም 6. የሰዉት ክብደት መቀነስ

7. ማቅለሽለሽ 8. ከፍተኛ የሆነ የራስ ምታት 9. መቅት 10 አላወቀወም 11 ሌላ ካለ

_____.

403. ¾+u= ug ¾T>Ã²¨< "Èƒ '¨<?

1. +u= ¾Á²¨< c¨< c=eM ¨ÃU c=Áe'Øc¨< u›¾' U¡"Áƒ 2. u Ï uSÚvuØ

3. cG"-‹" uÒ^ uSÖkU 4. u}SddÃ °n uSSÑw 5. ያልተፈላ ወተት በማጡጣት

6. K?L "K _____.

404. ¾+u= ug " "Èƒ SŸLŸM Ã‰LM?

1. uU"eMuƒ" uU"e'Øeuƒ Ñ>²? ›ó‹""›ó"Ý‹"" uSgð" 2. u Ï SÚvuØ"

uS}¨< 3. ሐ እቃዎችን በÒ^ ባለSÖkም 4. ¾u?ƒ Se¢„‹" uS´Òƒ 5. ጥ\ ¾›SÒÑw G<'@ 6. uìKAƒ 7.›L¨<k¨<U 8. K?L "K _____.

405. uGÑ^‹" ¾+u= ug Ù S\"I ¡U" ዋጋ U" ÁIM ’¨< ብለው ያስባሉ?

1. Ÿ¡õÁ 'í 2. ¡õÁ"<]S××¯ ’¨< 3. uSÖ'<U u=J" ¨<É ’¨<

406. የ ፀረ –ቲቢ መድሀኒትዎን ይወስዳሉን ? 1. ›- 2. ¾KU 3. ›MËS‘Ÿ<U

407. የ ፀረ –+u= SÉG'>ƒዎ" e"ƒ Ñ>²? ይወስዳሉ? 1. u¾k'< 2. udU"ƒ ›"É Ñ>²?

3. udU"ƒ G<Kƒ Ñ>²? 4. udU"ƒ feƒ Ñ>²? 5. K?L "K _____.

408. መቼ ነበር የ ፀረ ቲቢ በሽታ መድሀኒት መወሰድዎን ያቋረጡት?----------

409. በእርስዎ ›e}dcw'T¨¨< u+u= ug ¾T>Á²¨<? 1. T"—¨< c¨< 2. ÉG-‹ w‰

3. u?ƒ ¾K?L†¨< c-‹ w‰ 4. ›M¢M ›²¨<]-‹ w‰ 5. ›Å"³» ê }ÖnT>-‹

w‰ 6. Ÿ›?‹ ›Ã y=/›?Ée Ò‘ ¾T>•\ c-‹ w‰ 7. e‘ሀ ƒ ¾T>•\ c-‹ w‰

8.K?L "K _____.

410. ¾+u= ug SÇ" Ã‹LM"? 1. ›- 2. ¾KU

411. "Èƒ ’¨< ¾+u= ug ¾T>É¨< 1. uvIL© I¡U" 2. u?ƒ °[õƒ uTÉ[Ó/ÁK

I¡U" 3. በÖ?" ድርጅቶች ወስጥ የሚሰጥ የ ፀረ ቲቢ መድሀኒት 4. ›L¨<k¨<U 5. K?L "K _____.

¡õM ›Ueƒ:- eK +u= ›SK"Ÿƒ SÖÃp

501. የ +u= በሽታ Ãµ—M wKው ያስባሉን ? 1. ›- ' U¡"Á~U ----------

2. ¾KU ' U¡"Á~U ----------

502. uእርስዎ ›SK"Ÿƒ' የ +u= በሽታ ምን Á¡M ›eÑ> ’¨< ብለው ያስባሉ? 1. uxU ›eÑ>

2. uSÖ'< ¾T>ÁcÒ 3. በጣም አስጊ አይደለም

503. u›Ñ^‹"/ u¡ML‹" የ+u= በሽታ U" Á¡M ›cÑ> "¨< ይላሉ ? 1. u×U ›eጊ

2. uSÖ'< ¾T>ÁcÒ 3. u×U ›eÑ> ›ÃÅKU

504. ¾+u= ug uF Ã´ዎት ምን ያደርጋሉ? 1. õ^KG< 2. Å"Ñ×KG</ Ñ[TKG< 3. ›õ^KG<

4. "ÅÇKG</}eó u=e እሆናለሁ 5. K?L "K _____.

505. ¾+u= ug u=Ã´ዎት ለማን ይናገራሉ? 1. KGŸ=U/ KK?L Ö?" c^}— 2. KvM/ T>eƒ

3. Ku?}cw 4. Kp'w ÕÅ—Ã 5. KT"U ›L¨^U 6. K?L "K ___.

506. ¾+u= ug UM ¡ƒ u=cTዎት U" ያደርጋሉ? 1. ¨Å Ö?" }sTƒ H@ÇKG<2¨ Å SÉG'>ƒ u?ƒ H@ÇKG< 3. ¨Å vIL© SÉG'>ƒ ›ªm H@ÇKG<

4. ^c?" KTŸU V¡^KG</ upÖL pÖM 5. K?L "K ------.

507. eK +u= ug e ያስቡ በአብዛኛው ¾T>ÁeÚ"pዎት ሁኔታ U"É "¨<? ----------

¡õM eÉeƒ:- I¡U" KTÓ–ƒ ¾T>Ád¿ƒ" õLÑ<ƒ KT¨p ¾T>[Ç SÖÃp

601. u›w³—¨< ሲያምዎት የት ይሄዳሉ? 1. ¾ÓM Ö?" É'Ïƒ 2. ¾S"Óeƒ Ö?"

×u=Á/JeúM 3. vIL© Q¡U" ›ªm-‹ 4. S"Óe ©vM J'</uGÃT• ©¨ Å T>S^ Ö?" É'Ïƒ 5. K?L "K ----------.

602. e"ƒ Ñ>²? ¨Å Ö?" É'Ïƒ-‹ H@Åው ¡SΦM ? 1. u¯Sƒ G<Kƒ/Ÿ²=Á uLÃ

2. u›Sƒ ›"É Ñ>²? 3. በአመት Ÿ›"É Ñ>²? u ‹ Ó" u=Á"e vKóƒ ›Ueƒ አመት ሁለት

Ñ>²? 4. u›Ueƒ ›Sƒ ›"É Ñ>²? 5. u›Ueƒ ›Sƒ ¨<eØ ›MH@ÉŸ<U 6. K?L "K----

603. ¨Å Ö?" É'Ï„‹ "Mሄዱ U¡"Áƒዎ ምንድን ነው? 1. ¾ƒ "ÅUH@É ›L¨<pU

2. ¡õÁ 3. ¾ሙÕÕጉ‹Ó'/ŸÖ?" É'Ï~ ÁK¨< 'kƒ 4. uQ¡U" vKVÁ-‹ U'ƒ

¾K¯U 5. ¾I¡U“ vKVÅ-‹ ›SK”Ÿƒ ›ÁeÅe}¯U

¡õK cvƒ:- ¾+u= Ó”³u?“ ¾S[Í U”à‹

701. eK +u= ug umÓ” ³u? አግኝቻለሁ ብለው ያስባሉን? 1. ›- 2. ¾KU

702. eK +u= ug umÓ” ³u? KTÓ–ƒ ይV¡^ሉን ? 1. ›- 2. ¾KU

703. eK +u= ug S[İ KSeÖƒ uIw[}cu< ²”É uØ\ G<’@ ¾S[Í U”ß

U”É”¨< wKውያስባሉ? (fe~” ª“ ª“ ¾S[Í U”ß ጥቀሱ) 1. ከጋዜጣ፣ ከመጽሄት፣ 2. ከራዲዮ

3. ከቴK?y=¶” 4. ŸTe ¨mÁdK? Ç 5. Ÿበራሪ êOፎች 5. ’e°L© SÓKÝ

6. ŸÖ?“ c^}™‹ 7. Ÿu?}cw’ÕÅ—’Ñ<[u?ƒ 8. ŸGÃT• ©S] -‹ 9. ŸSUI^”

10. ŸK?KA‹ _____.

u³=l Ø“ƒ uSd}õ eK}vu^‹G<˜ u×U ›ScÓ“KG< ::

በጥናቱ ለሚሳተፉ ተሳታፊዎች የሚሰጥ መረጃ

ስሜ አህመድ እስማኤል ይባላል፡፡ በአዲስ አበባ ዩኒቨርስቲ በህክምና ላብራቶሪ ትምህርት ቤት የክሊኒካል ላብራቶሪ ሳይንስ የድህረ ምረቃ ተማሪ ነኝ፡፡የጥናቱ ዓላማም የቲቢ በሽታ ለፀረ-ቲቢ መድሀኒቶች የሚያሳየውን

አይበገሬነት በአዲስ የቲቢ መድሀኒት ጀሚሪዎችና ከዚህ በፊት የቲቢ መድሀኒት ተጠቅመው ለሚያውቁ ህመሟን ላይ ያለውን ስርጭት እና ይህ የቲቢ በሽታ ለፀረ-ቲቢ መድሀኒቶች ያለው አይበገሬነት ከቲቢ በሽታ ህመሟን እውቀት፣ አመላካከት እና፣ ህመሟኖቹ ከሚያደርጉት ልዩ ልዩ ተግባሮች ጋር ያለውን ግንኙነት በምስራቃዊ አማራ ክልል ለማጥናት ነው፡፡ የላብራቶሪው ምርመራውየሚከሄደውአዲስ አበባ በሚገኘውየኢትዮጲያ ጠፍና ስነ ምግብ ምርመራ ተቋም ሲሆን ጥናቱ የሚከሄደው ከታካሚዎች መካከል በአክታቸው ላይ የቲቢ ጀርም የተገኘባቸውንናያልተገኘባቸውን በመወሰድ ነው፡፡ እርስዎ የሚሰጡት መረጃ ደግሞ በቀጣይ የቲቢ በሽታን ለመከላከል እና አስፈላጊውን የመከላከያ ዘዴ ለመንደፍ የሚደረገውን ጥረት ለማገዝ ይረዳል፡፡ እርስዎ የሚሰጡት መልስም ለማንም ይፋ አይሆንም ሚስጥርም ሆኖ ይያዛል፡፡ የእርስዎ ስም በቃለ መጠይቁ ላይ አይፃፍም፡፡ የእርስዎ ተሳትፎም በፍቃደኝነት ላይ የተመሰረተ ሲሆን ቃለ መጠይቁ ለማቋረጥ ቢፈልጉ በጠፍ ድርጅቱ ከሚያገኙት ማንኛውም አገልግሎት ጋር ግንኙነት አይኖረውም፡፡ በተጨማሪም እርስዎ በጥናቱ በሚሳተፉበት ወቅት ምንም አይነት እርስዎን የማንዳ ሁኔታ አይኖርም፡፡ የሚሰጡትም እንደጠፍ መያተኛው ትዕዛዝ ሶስት ተከታታይ አክታ ሲሆን እያንዳንዱ ጥያቄ የሚወስደው ከ 10-15 ደቂቃ ብቻ ነው፡፡ በጥናቱ ሜጨረሻም የጥናቱ ወጠት ለእያንዳንድ የቲቢ ታካሚ በጠፍ ደርጅቱ በኩል የምርመራ ወጠቱ ይፋ ይደረግለታል፡፡ እንዲሁም ከነዚህ ታካሚዎች መካከል ለፀረ-ቲቢ መድሀኒቶች አይበገሬነት ያሳዩ ህመሟን ለሚመላከተው አካል በማሳወቅ ህክምና እንዲያገኙ ይደረጋል፡፡ ለጥናቱ ስኬታማነት ትክክለኛ መረጃ ይሰጠን ዘንድ በአክብሮት እንጠይቃለን፡፡

ስለተባበራችሁኝ አመሰግናለሁ :: nK SÖÃl" ÃkØK<

Ø“~” ¾T>ÁŸH>Å¨< vKS<Á eMj lØ‘ 0913 68 13 99¾›Ã›‘u= eMj lØ‘ :251-115 538734

ለጥናቱ ተሳታፊዎች የተዘጋጀ የፍቃደኝነት መግለጫቅፅ (ኮንሰት)

እኔ ---------- uዚl ጥ ናት ውስጥ ለ መሳተፍ በቂ ገለፃ ስለተደረገልኝ ሶስት ተከታታይ አክታ uጠፍ ባለመያው ትዕዛዝ መሰረት ለመሰጠትና ቃለ መጠይቁን የሚያካሂደው ባለመያ የሚጠይቀኝን ቃለ መጠይቅ ለመመለስ ፍቃደኝነቴን እገልፃለሁ፡፡ በጥናቱ ወቅትም ከመጀመሪያ እስከመጨረሻ ምንም አይነት እኔን የሚነዳ ሁኔታ እንደሌለ ተረድቻለሁ፡፡ ጥናቱም ለእኔ ብቻ ሳይሆን ለሌሎች በቲቢ በሽታ ለተያዙ ህመሟን ሁሉ እንደሚጠቅም ተረድቻለሁ፡፡

የፀረ ቲቢ መድሀኒቶችም በእነዚህ ጠፍ ድርጅቶች ወስጥ እንደሚኙ አወቄአለሁ፡ ፡ ጥናቱምበሜጨረሻ ጊዜ ለጥናቱ አለሜኽ ለሚመለከታቸውአካላት ብቻ ይፋ እንደሚሆን ተረድቻለሁ፡ ፡

ፊርማ ቀን **----/ ----/----**

በዚህ ጠቃሚጥናት ስለተባበራችሁኝ አመሰግናለሁ፡ ፡

ማሳሰቢያ፡ -ስለጥናቱ ተጨማሪ መረጃ ከፈለጉ በነዚህ ስልክ ቁጥሮች ይደውሉ Ø“~” ¾T>ÁŸH>Å¨< vKS<Á M¡lØ‘ 0913 68 13 99 ›Ã›‘u= eM¡ lØ‘: 251-115 538734

Annex V-Laboratory procedure

1. Sputum collection

1.1. Procedure

- ❖ Give the patient confidence by explaining to him/her the reason for sputum collection
- ❖ Instruct the patient to rinse his/her mouth with water before producing the specimen. This will help to remove food and any contaminating bacteria in the mouth

❖ Instruct the patient to take two deep breaths, holding the breath for a few seconds after each inhalation and then exhaling slowly. Ask him/her to breathe in a third time and then forcefully blow the air out. Ask him/her to breathe in again and then cough. This should produce a specimen from deep in the lungs. Ask the patient to hold the sputum container close to the lips and to spit into it gently after a productive cough. Sputum is frequently thick and mucoid, but it may be fluid, with chunks of dead tissue from a lesion in the lung. The colour may be a dull white or a dull light green. Bloody specimens will be red or brown. Thin, clear saliva or nasopharyngeal discharge is not sputum and is of little diagnostic value for tuberculosis.

❖ If the sputum is insufficient encourage the patient to cough again until a satisfactory specimen is obtained. Remember that many patients cannot produce sputum from deep in the respiratory track in a few minutes. Give him/her sufficient time to produce an expectoration, which s/he feels, is produced by a deep cough.

❖ If there is no expectoration, consider the container used and dispose of it in the appropriate manner.

❖ Check that the container is securely closed and label the container (not the lid) clearly

❖ Wash hands with soap and water

❖ Give the patient a new sputum container and make sure that s/he understands that a specimen must be produced as soon as s/he wakes up in the morning

❖ Demonstrate to the patient how the container should be securely closed

❖ Instruct the patient to bring the specimen back to the health center or laboratory

1.2. **Ziehl Neelsen methods: Procedure**

Prepare smear as describes; allow air to dry

➢ Heat fix smear either on an electrics slide warmer at 65 to 75 ^{oc} for at least 2 hours or pass slide through Bunsen burner flame as for other bacteriological smear. Don't over heat

- Cover smear with a 2x 3 cm piece of filter paper to holds the stain on the slide and to minimize precipitation of dye crystal on to the smear
- Flood the paper strip with carbool fuchsine
- Heat the slides to steaming with Bunsen burner or an staining electric racks, let stands 5 minutes .if the smear dries, add more stain add more stain but don't re heat.
- Use forceps to remove paper strips from slides and to place them in discard containers wash slides with water (use tap water or water from reservoir bottles)
- Flood smear with acid alcohol, allow to destain for 2 minutes
- Wash smear with again with water and drain
- Floods slides with mthylene blue and counter stain for 1 to 2 minutes
- Rinse with water, drain 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: 4% and Phosphate buffer 0.067M, pH 6.8

Procedure:

Step1-Weight 4g in 100 ml distill water

Step2- Weight 2.97 g in 100 ml distill water

Step 3- Mix step 1 &2

Step 4- Add 0.5g NALC

Sputum processing

Step 1- transfer the sputum (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 deposit on to two slopes of LJ medium labeled with the ID number

Step12-use a pipette inoculate each slope with 3 to 4 drops

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

3. Preparation of egg-based LJ media

LJ medium containing glycerol favors the growth of M. tuberculosis while LJ medium without glycerol but containing pyruvate encourages the growth of M. bovis. Both should be used in countries or regions where patients may be infected with either organism. And LJ medium prepared according to EHNRI standards.

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

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.

3.1. **Quality Control**

3.1. **Sensitivity of plain egg based medium.**

Serious problems affecting the sensitivity of culture medium, i.e. its capacity to sustain consistent growth of tubercle bacilli, can be detected 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

4. Main biochemical tests to identify M. tuberculosis

A). **Niacin accumulation test.** Nicotinic acid or niacin is produced by all Mycobacteria, but some species, such as M. tuberculosis, Mycobacterium simiae and M. bovis BCG excrete it due to a blockade in their scavenging pathway. The excreted niacin accumulates in the culture medium and is evidenced in the presence of cyanogen halide with a primary amine. Niacin-negative M. tuberculosis strains are extremely rare.

B). **Growth in the presence of p-nitro benzoic acid.** This compound inhibits the growth of several species in the M. tuberculosis complex: M. tuberculosis, M. bovis, M.africanum, and M. microti (Tsukamura 1984, Leão 2004).

C). **Nitrate reduction test.** This test is particularly useful for differentiating M. tuberculosis, which gives a positive reaction, from M. bovis, which is negative (Tsukamura 1984, Vincent 2003).

D). **Catalase test.** Catalase is an intracellular enzyme that transforms hydrogen Peroxide to oxygen and water. The 68°C catalase is a heat-tolerance test measuring the catalase activity at high temperature. Characteristically, M. tuberculosis gives negative results, as do other species in the M. tuberculosis complex. (Vincent 2003)..

5). SOP for drug susceptibility testing, proportion methods

Principle(s) of Procedure: The proportion method (Canetti, modified) determines the percentage of growth (number of colonies) of a defined inoculums on a drug-free control medium versus growth on culture media containing the critical concentration of an anti-tuberculosis drug.

1. **Preparation of LJ drug containing media**

Isoniazid: weight 0.2 µg/ml, for the dry and pure INH, the correction factor 1

- Measure 500ml of LJ solution in to flask

- Dissolved 0.1 g INH in 10 ml sterile water (stock solution I)
- Add 1.0 ml solution I to 9 ml sterile water(solution II)
- Add 1 ml solution II to 9 ml sterile water (solution III)
- Add 1 ml of solution III in to 500 ml of LJ solution

Ethambutol: the correction factor for EMB is 1

- measure 500 ml of LJ solution in to flask
- dissolved 0.1 g EMB in 10 ml sterile water (stock solution I
- Add 1 ml solution to 9 ml sterile water (solution II
- Add 1 ml of solution II in to 500 ml of LJ solution

Dihydro-strptomycin: as sulphate , dry and pure the factor is 1.251

- measure 500 ml of LJ solution in to flask
- 0.125 g SM in 10 ml sterile water (stock solution I)
- add 1 ml of solution I to 9 ml sterile water (solution II)
- add 2 ml of solution II in to 500 ml of LJ solution

Rifampicin (RMP): for RMP usually the correction factor is 1 for a pure RMP

- measure 500 ml of LJ solution in to the flask
- dissolve 0.02 g RMP in 2.0 ml with DMSO in 50 ml tube (solution I)
- add 18 ml of distill water to the solution I (solution II)
- Add the solution II to 500 ml LJ media

Preparation of bacterial suspension

- Use a sterile, small thick walled screw capped glass tube containing 5-7 glass beads
- Add 2 drops of sterile saline or distilled water
- With a loop , scrape colonies from all over the culture strain (try to pick up portions from all colonies)
- Shake gently the loop over the beads vortex it

- Let stand for 15-30 minutes to allow the larger aggregates bacteria to settle
- Add 4 ml of sterile saline or distilled water
- The homogenous upper part of the supernatant should be aseptically transferred to another tube with similar dimensions of the McFarland tube for comparative purposes
- The bacterial suspensions are adjusted with sterile distilled water to a turbidity matching a McFarland standard No 1.

2. **Dilution steps**

- Measure 4.5 ml of sterile saline or distilled water in four tube
- Add 0.5 ml of bacterial suspension which is adjusted to McFarland standard No 1 to the first tube (10^{-1})
- Add 0.5 ml of 10^{-1} dilution to the second tube (10^{-2})
- Add 0.5 ml of 10^{-2} dilution to the third tube (10^{-3})
- Add 0.5 ml of 10^{-3} dilution to the last tube (10^{-4})

3. **Inoculation**

- Make all sets of culture media properly with patient`s identification
- The inoculation may performed with pipettes or a calibrated loop (10 µl).
- Care has to be taken to distribute the inoculums evenly over the lower 80 % of the culture media
- Dilutions 10^{-2} (suspension 1) and 10^{-4} (suspension 3) are required for inoculums
- Test media containing the drug INH, RMP, DSM, EMB are inoculated with dilutions 10^{-2} (suspension1)
- For two growth control media from the same bach are inoculated with 10^{-2} (susepension 1) and 10^{-4}(suspension 3)

4. **Incubation**:

- The incubation temperature shall be 35-37^{o}c
- The seeded media are examined for contamination after one week of incubation

- The first reading of drug susceptibility test results is done at four weeks of incubation
- At the time all strains showing drug resistance can be reported as drug resistance
- The last reading of drug susceptibility test result is done at six weeks of incubation

Calculation: % resistance= number of colony on drug /number of colony on control*100

Slants have to be read after 4 weeks of incubation, as provisional result and after 6 weeks of incubation for the definitive interpretation of results

Report: no growth=0, Confluent=3^+, more than 100 colonies =2^+ , recorded actual number of colonies=1-100 colonies, Susceptible : no growth or growth < 1%, resistance=>= 1% growth

7. 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.