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EPIDEMIOLOGY AND DRUG RESISTANCE PATTERN OF *MYCOBACTERIUM TUBERCULOSIS* IN NORTH WEST ETHIOPIA: RESOURCE LIMITED SETTINGS

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ABBREVIATIONS

AFB	Acid Fast Bacilli
***ahp*C**	Gene encodes alkyl hydro peroxide reductase
AHRI	Armauer Hansen Research Institute
AIDS	Acquired Immunodeficiency Syndrome
AMK	Amikacin
BSL-2	Bio safety level -2
Cs	Cycloserine
DNA	Deoxyribonucleic Acid
DOTS	Directly Observed Treatment Short-Course
DR	Direct repeat
DST	Drug Susceptibility Testing
EHNRI	Ethiopian Health and Nutrition Institute
EMB	Ethambutol
***emb*B**	arabinosyl transferase
***emb*CAB**	Operon encoding for mycobacterial arabinosyl transferase
Eto	Ethionamide
HBC	High Burden Countries
HIV	Human Immunodeficiency Virus
INH	Isoniazid
***inh*A**	Enzyme enoyl reductase encoding gene
IUATLD	International Union Against Tuberculosis and Lung diseases
KAN	Kanamycin
KAP	Knowledge Attitude and Practice
***Kat*G**	Encodes catalase peroxidase gene
Lfx	Levofloxacin
LJ	*Löwenstein-Jensen*

MDR-TB	Multidrug resistant tuberculosis
MGIT	Mycobacteria Growth Indicator Tube
MIRU	Mycobacterial Interspersed Repetitive Units
MOTT	Mycobacterium other than tuberculosis
NALC-NaOH	N-acetyl L-cysteine- sodium hydroxide
PCR	Polymerase Chain Reaction
***pnc*A**	Enzyme pyrazinamidase encoding gene
PTB	Pulmonary tuberculosis
PZA	Pyrazinamide
RD	Region of difference
RFLP	Restriction fragment length polymorphisms
RIF	Rifampicin
rpoB	β-subunit of RNA polymerase
rpsL	S12 ribosomal protein
rRNA	Ribosomal Ribonucleic Acid
Rrs	16S rRNA
SNP	Single Nucleotide Polymorphism
STM	Streptomycin
TNFα	Tumor Necrosis Factor alpha
VNTR	Variable Number Tandem Repeats
WHO	World Health Organization
XDR	Extensively Drug Resistant

ABSTRACT

Background: Tuberculosis (TB) remains a major global health problem and ranks as the second leading cause of death among deaths caused by infectious diseases worldwide. Despite the availability of short course regimens as first line anti tuberculosis drugs, emergence of drug resistant *Mycobacterium tuberculosis* strains pose a major challenge to the prevention and control efforts of national tuberculosis programs (NTPs). In developing countries, including Ethiopia, the burden of tuberculosis and /or drug resistance profile of *M.tuberculosis* remains largely unexplored, mainly due to lack of quality controlled second line laboratory tests.

Objectives: To assess the mycobacterial genotypic diversity and drug resistance pattern of *M. tuberculosis* complex in North West Ethiopia: resource limited settings. Moreover, it was to assess the knowledge, attitude and practice (KAP) of TB suspects towards tuberculosis

Methods: Health care institution-based cross-sectional study was designed and conducted to assess: 1) Mycobacterial strain diversity 2) drug resistance pattern of *Mycobacterium tuberculosis* and 3) to evaluate the KAP of TB suspects towards TB in North West Ethiopia: resource limited settings. Study Subjects from health facilities in Benishangul Gumuz region and Awi zone of Amhara region who consented to participate were included in the study. 112 Smear positive samples were collected from study participants, stored and transported to Armauer Hansen Research Institute (AHRI) TB laboratory. At AHRI laboratory *Lowenstein–Jensen* (LJ) culture, drug susceptibility testing, RD9 deletion typing and Spoligotyping were performed from April 2013 through June 2014. Drug susceptibility testing (DST) was done by the proportion method on 24 well plates using 7H10 medium. The isolates were further characterized by spoligotyping and compared with updated SpolDB4.0 database to identify the circulating strains. Information for KAP study was collected on well-structured questionnaires. SPSS V.20 statistical software was used for data entry, data cleaning and analysis.

Results: DST result of isolates from 87 samples showed one or more drug resistance in 16.5% with higher rate of monoresistance to isoniazid (6.9%). Multidrug resistance of 2(2.3%) was observed with 1(1.3%) and 1(8.3%) distribution among new and retreatment cases respectively.

From 75 *Mycobacterium tuberculosis* isolates typed, 39 particulars spoligotype patterns with 32(42.7%) of "New" isolates was observed. SIT289, SIT53, SIT149, SIT37 and SIT134 were the

predominant strains circulating in the study region with proportion of 14.7%, 12.0%, 8.0%, 5.3% and 4.0% respectively

From 383 participants involved for KAP study 231(60.3%) were male and 152(39.7%) were female. Most frequent respondents' sources of information about TB were health professionals in 131(34.2%) and 20(5.2%) had never heard about tuberculosis. 254(66.3%) knew that TB transmission is aerosol while 81(21.1%) do not know about it. 223(58.2%) responded TB transmission can be prevented by covering mouth and nose while coughing and sneezing but 125(32.6%) do not know about means of TB prevention. Respondents' lack of knowledge 169(49.6%) and 63(18.5%) were reasoned accessibility and affordability for their delay in health service seeking behavior.

Conclusion: Although current level of drug resistance appears to be low, the risk for a sudden increase is high because of the relatively higher monoresistance to INH. It is essential to maintain high quality DOTS in the area to keep MDR low.

Majority of respondents also had poor knowledge and several misconceptions about TB that needs to be addressed through continuous health education on cause, transmission, prevention, treatment and services available.

Key words: *Mycobacterium tuberculosis*, Strain diversity, Drug resistance, KAP, Benishangul Gumuz region, Northwest Ethiopia

CHAPTER ONE: INTRODUCTION

Tuberculosis (TB) i s an i nfectious d isease cau sed b y t he b acillus *Mycobacterium t uberculosis* complex. It t ypically a ffects th e lu ngs (pulmonary TB) b ut c an a ffect o ther s ites a s w ell (extra pulmonary TB). The disease is transmitted through aerosol infection when people who are sick with pulmonary TB expel bacteria. TB remains a major global health problem. It causes ill health among millions of people each year and ranks as the second leading cause of death from infectious diseases worldwide, a fter t he hu man i mmunodeficiency virus (HIV). A ccording to t he 2013 W orld H ealth Organization (WHO) global tuberculosis report, in 2012 there were 8.6 million cases developed TB and 1 .3 millio n d ied f rom th e d isease. T his is d espite th e availability o f tr eatment th at w ill c ure most cases of TB. Short course regimens of first line drugs that can cure around 90% of cases have been available since the 1980s (WHO, 2013).

In d eveloping c ountries m ore pe ople a re d ying of T B t han a ny ot her i nfectious di seases. It comprises 25% of all avoidable deaths. Nearly 95% of all TB cases and 98% deaths due to TB are in developing countries and 75% TB cases are in productive age groups (WHO, 2009).

Ethiopia is one among the ten countries with triple high burden (TB, HIV, and MDR TB) countries in t he w orld a nd i t i s a mong t hree c ountries i n A frica. In 2012 E thiopia ha s t he pr evalence a nd incidence of TB of 224 and 247 per 100 000 popu lation respectively. In the same year there were a total of 147,592 TB cases notified (143,503 new and 4089 retreatment) with an estimated 1600 new and 480 r etreatment c ases of M DR T B but onl y 469 (<1%) ne w a nd 18 0 (4%) r etreatment c ases tested f or M DR of w hich onl y 30 c ase a nd 10 2 c ases w ere l aboratory c onfirmed respectively (WHO, 2013).

Drug resistance in TB threatens the National Tuberculosis Control Programme in several countries, and th e ma jor p roblem is mu ltidrug r esistance TB (MDR-TB) (Aziz *et a l.*, 2006) . M DR-TB i s defined as *M. tuberculosis* strains that are resistant to at least isoniazid (INH) and rifampicin (RIF), the two key first line drugs in short course TB chemotherapy. Resistance to any single TB drug is close to 10% in most African countries (WHO, 2006b). Recently, extensively drug resistant (XDR) *M. t uberculosis* (defined a s r esistant to a t le ast INH, RIF, a nd f luoroquinolone, a nd e ither aminoglycosides (amikacin, ka namycin or c apreomycin or bot h) i s emerging (Demissie *et a l.*, 1997).

Globally in 2011, there were an estimated 630 000 cases of MDR-TB (460 000– 790 000) among the world's 12 million prevalent cases of TB. 310 000 (220 000–400 000) MDR-TB cases were among notified TB patients with pulmonary TB. The proportion among new and retreatment cases showed high proportion among previously treated cases which is 3.7% (2.1–5.2%) of new cases and 20% (13–26%) of previously treated cases are estimated to have MDR-TB. According to WHO global TB control program, Ethiopia is one of the 27 high burden MDR-TB countries with more than 5000 estimated MDR-TB patients annually (WHO, 2012).

The effective control of TB is based on the immediate detection of *M. tuberculosis*, followed by the prompt implementation of adequate anti-TB therapy (Coll *et al.*, 2005). The emergence of strains resistant to the major anti-TB drugs speeds up the need for rapid methods for the identification of resistant *M. tuberculosis* strains in order to treat the disease effectively and, at the same time, to prevent the spread of resistant strains (Drobniewski *et al.*, 2007, Gali *et al.*, 2006, Gali *et al.*, 2003, Jacobs *et al.*, 1993). MDR *M. tuberculosis* strains have emerged worldwide and seriously threaten TB control and prevention programs (WHO, 2006a).

One of the aims of ensuring effective management of TB is to minimize the development of drug resistance, which results from inadequate therapy. Surveillance of anti-TB drug resistance is therefore an essential tool for monitoring the effectiveness of TB control programs and improving control efforts. The emergence of drug resistance in recent years has highlighted the importance of an effective control strategy for TB. The rapid spread of drug resistance, MDR-TB and XDR-TB, both in new and in previously treated cases, adds urgency to the need for decisive action to develop and implement control measures (Jain and Mondal, 2008).

1.1. LITERATURE REVIEW

1.1.1. Etiology

TB is an infectious disease caused by *M. tuberculosis* complex (MTC) bacteria which has an endemic character and worldwide distribution. The MTC comprises closely related species responsible for strictly human and zoonotic TB. The complex consists of seven species and subspecies including *M. tuberculosis*, *M. Canetti*, *M. africanum*, *M. pinnipedii*, *M.microti*, *M. caprae* and *M. bovis*. Despite the different species tropisms, the MTC is characterized by 99.9% or

greater similarity at the nucleotide level and possess identical 16S rRNA sequence (Dye *et al.*, 2005).

M. tuberculosis is a large non motile and non-spore forming rod shaped bacillus grouped under the order actinomycetales. The bacteria in the group are known for the high lipid content in their cell wall. The cell wall is the most distinctive anatomical feature of the bacteria. It is constituted by an inner peptidoglycan layer which seems to be responsible for the shape forming property and the structural integrity of the bacterium. The lipid coat confers the distinctive characteristics of the group: acid fastness, extreme hydrophobicity, resistance to weak disinfectants. It probably also contributes to the slow growth rate of some species by restricting the uptake of nutrients (Draper, 1998, Murray P., 2005, Ryan, 2004).

M. tuberculosis strains can be classified into a number of major clades according to defined evolutionary markers. It is hypothesized that strains comprising these clades have evolved different properties which may influence a local strain population structure. This evolved different properties growth was exclusively attributable to drug susceptible strains. Recent evidence suggests that these differences likely reflect enhanced pathogenicity rather than transmissibility. The rapid emergence of different strains demonstrates adaptation to conditions within the study community and poses a grave challenge to future TB control (van der Spuy *et al.*, 2009).

Although TB may manifest itself at any tissue, the lung represents the main port of entry and is an important site for disease manifestation (Murray P., 2005).

1.1.2. Risk Factors

Risk factors that accelerate TB infection include: poverty, changing demographics with increasing crowding and changing age structure (children less than five years and the elderly greater than 65 years are more vulnerable) (Pena *et al.*, 2003). Other factors such as genetic disposition, inadequate health coverage, chronic infections (HIV/ AIDS, Diabetes mellitus, renal disease, lung damage and various malignancies) enhance development of the disease (Perenboom *et al.*, 1995). Neglect and under funding of TB control programmes, previous exposure to mycobacterial infections, protein energy malnutrition, and cytotoxic therapy can also aggravate disease progression (Cantwell and Binkin, 1997).

Accessing for effective TB services can be affected by Economical, geographical, sociocultural, and health system barriers. Many of the poor and vulnerable groups encounter more than one of these overlapping sets of barriers and have greater difficulty in overcoming them (WHO, 2005).

1.1.3. Pathogenesis

Healthy adults exposed to relatively low numbers of bacteria generally clears them before considerable damage to the lung. But if the phagocytic cells do not clear the infection, new T cells, polymorphonuclear cells (PMNs) and more macrophages continue to be attracted to the area where bacteria are growing (Udoh, 2009).

Mycobacteria may also be transported to other organs via the lymph vessels or bloodstream and produce dissemination foci there. The host eventually develops granulomas, foci fibrose, scar, and calcify but the infection remains clinically silent. In about 10% of infected persons the primary TB reactivates to become organ TB either within months or after a number of years. Reactivation begins with a caseation necrosis in the center of the granulomas (also called tubercles) that may progress to cavitation and frequently stems from old foci in the lung apices. Tissue destruction is caused by cytokines among which tumor necrosis factor alpha (TNFα) appears to play an important role. These cytokines are also responsible for the cachexia associated with tuberculosis. The body's immune defences have a hard time in containing necrotic tissue lesions in which large numbers of *Mycobacterium* cells occur (Kayser, 2005).

In some cases where the phagocytes fail to kill the bacteria, the T cells and macrophages wall off the growing lesion with a thick fibrin coat. The walled off lesion is called tubercle. Tubercles eventually calcify, giving rise to the hard-edged lesions visible in chest x-rays. Phagocytes unsuccessfully trying to kill the bacteria cause considerable damage to lung tissue by releasing lysosomal enzymes and by producing TNF. Initially the areas where bacteria are growing have a thick, cheese like appearance (caseous necrosis). As bacteria continue to grow and phagocytes continue to enter the area, the necrotic region becomes much more liquid. A person with liquefied lesions is more contagious than lesions in caseous necrosis (Sudre *et al.*, 1992).

1.1.4. Transmission

The main reservoir of *M. tuberculosis* is the patient with pulmonary TB (PTB). Such patients may have pulmonary cavities that are rich in bacilli. Patients with cavitary PTB are almost always

smear positives and are the main source of infection in the transmission of TB. The number of infectious droplets projected into the atmosphere by a patient is very high when coughing or sneezing. When they come into contact with the air these droplets rapidly dry and become very light particles but still containing live bacilli that remain suspended in the air. In an enclosed space, the droplets can remain suspended for a long time and the bacilli remain alive for several hours in the dark. When a person inhales these infectious particles, the large particles are deposited on the mucous of the nasopharynx or the trachea-bronchial tree and are expelled by mucociliary clearance. But the smallest particles having less than a few microns in diameter can penetrate to the alveoli. The closer and the more prolonged the contact with an infectious patient is the greater the risk of infection as this is linked to the density of the bacilli in the air the individual breathes and the amount of the air inhaled (WHO, 2003).

In general, TB spreads through droplets, which are expelled when persons infected with the bacilli cough, sneeze or sing (Nachega and Chaisson, 2003). Inadequate infection control measures will contribute for the ongoing transmission of TB, MDR-TB and XDR-TB in health care facilities and congregate settings (Nodieva *et al.*, 2010).

1.1.5. Clinical Manifestations

The most common clinical manifestation of TB is pulmonary distress which is slow and cleverly indirect at onset. Patients typically have nonspecific complaints of malaise, fever, weight loss, Cough, shortness of breath, chest pain and night sweating. Sputum may be bloody if cavitary and purulent (Fauci S., 2008).

Disseminated TB is defined as involvement of many organs simultaneously and can occur as a result of primary progressive disease or reactivation of latent infection. The clinical manifestation of pulmonary involvement is a miliary pattern rather than an infiltrate in most cases. Not all patients with disseminated disease have pulmonary involvement. Miliary TB accounts for about 1–2% of all cases of TB and about 8% of all forms of extra pulmonary TB in immunocompetent individuals. The disease is more frequently encountered in immunosuppressed individuals. Mortality is high despite chemotherapy and may be related to delays in diagnosis and other commonly present underlying medical conditions (Sharma *et al.*, 2005).

1.1.6. Drug Resistant and Multi-Drug Resistant Tuberculosis

Anti-TB 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 (Johnson *et al.*, 2006). Drug resistance in TB causes the decrease in the susceptibility to anti-TB drugs for *Mycobacterium* strains of human type that have never had contact with the drugs (Mitchison, 1984). Drug resistance in tuberculosis is the result of spontaneous mutation as well as poor programmatic and individual care performance (Gillespie, 2002).

The use of combination of drugs for prevention of TB faced challenges due to the emergence of MDR-TB. Among the different patterns of drug resistance, multidrug resistance has been clearly identified as a severe form of TB that requires prolonged duration of treatment, high cost and severe side effects. MDR-TB has a strong impact on morbidity and mortality (WHO, 2009).

The burden of MDR-TB in Africa remains largely unexplored, mainly due to lack of quality controlled second line testing. The proportion of MDR-TB, as has been shown by some African countries, seems relatively low with a frequency ranging from 0.5% to 3.9% among new TB cases and 0.0% to 16.7% among previously treated TB patients. Inadequate laboratory capacity to perform diagnostic testing among TB patients and barriers to conducting drug resistance surveys could be one of the reasons for the absence of representative data (WHO, 2010).

In the WHO global tuberculosis control report, the prevalence of MDR-TB in Ethiopia was estimated to be 1.6% among newly diagnosed cases and 12% among retreatment cases which is projected from a single national survey of 2005 (WHO, 2013). In recent studies conducted at St Peter's TB Specialized Hospital and Ethiopian Health and Nutrition Research Institute (EHNRI), the prevalence of MDR-TB was found to be 4.3% (Agonafir *et al.*, 2010) and 46.3% among retreatment cases (Abate *et al.*, 2012); this shows an existence of higher percentage of MDR-TB in Ethiopia, particularly in retreatment cases, compared to WHO report which is only 12% (WHO, 2013).

To confront MDR-TB, it is also necessary to increase our willingness to detect and treat it through improving strategies focusing on more rapid diagnosis, better access to drugs, adherence to treatment, knowledge on infection prevention and decentralization of primary care level services to patient centered approach, and community-based support (Goemaere *et al.*, 2007).

1.1.7. Molecular Mechanism and Genetic Basis of Drug Resistance

During bacterial multiplication, resistant bacilli evolve through spontaneous mutation and with defined frequency. Mutation that results in INH resistance to *M. tuberculosis*, for example, occurs at a rate of 10^{-7} to 10^{-9} per cell division and leads to an estimated resistance of 1 in 10^{6} bacilli in drug free environment (Lambregts-van Weezenbeek *et al.*, 1998).

Exposure to a single anti-TB drug due to irregular intake, poor drug quality, inappropriate prescription and/or poor adherence to treatment could result in non-functional monotherapy. This will suppress the growth of bacilli susceptible to that drug but permits the multiplication of drug resistant organisms (Crofton and Mitchison, 1948, Mitchison, 1950).

The genetic basis of resistance to most anti-TB drugs is established. Resistance to RIF results from missense mutations in the *rpo*B gene, which encodes the β subunit of RNA polymerase (Miller *et al.*, 1994). RNA polymerase is an essential enzyme with five subunits that catalyzes the process of transcription. RIF specifically binds to the β subunit and prevents early steps of transcription that leads to the bacterial death. However, mutation in *rpo*B gene results in resistance by decreasing RIF binding affinity. Mutation leading to resistance of *M. tuberculosis* to RIF is rare and occurs at a rate of 10^{-10} per cell division with an estimated prevalence of 1 in 10^{8} cells in drug free environment. However, it rapidly results in the selection of mutants that are resistant to other anti-TB drugs (Goble *et al.*, 1993). Most commonly, it exists in conjunction with INH resistance, and thus defines a strain as being MDR (Turett *et al.*, 1995).

INH is a potent drug that inhibits the synthesis of mycolic acids in *M. tuberculosis*. It is a pro drug that becomes active when catalyzed by the enzyme catalase peroxidase. At least three genes, *kat*G, *inh*A and *ahp*C are involved in resistance to INH. Among these genes, *kat*G that encodes catalase peroxidase is mainly responsible for INH resistance. Approximately 50% of resistant isolates contain mutations in *kat*G with the majority localized to codon 315. It is found that the *kat*G gene encoding catalase peroxidase is defective in many INH resistant strains (Zhang and Yew, 2009).

Pyrazinamide (PZA) is also a pro drug that can be converted into an active form presumably by pyrazinamidase enzyme of susceptible organisms. The target for the active drug is not fully known. However, mutations in the gene *pnc*A, encoding for the enzyme pyrazinamidase are the major causes of PZA resistance. Between 72% and 98% of PZA resistance in clinical isolates is correlated

with mutations scattered throughout the 558 bp *pnc*A coding region and 11 promoter regions. The rate of mutation for PZA is 10^{-3} with a probability of resistance 1 in 10^{6} bacilli (Scorpio and Zhang, 1996).

Streptomycin (STM) is an antibiotic that interferes with prokaryotic protein synthesis. Resistance to it is mainly due to mutations in the *rps*L locus encoding the S12 ribosomal protein. Approximately 60% of STM resistant clinical isolates show *rps*L mutation. About 10% of resistant strains have mutations in 16S ribosomal RNA which is encoded by *rrs* gene. The mutation rate for STM is 10^{-8} resulting in resistance 1 in 10^{7} bacilli (Bottger and Sander, 1999).

Ethambutol (EMB) is a drug, which targets cell wall synthesis. The major mechanism of acquisition of resistance to the drug is associated with point mutations in the *emb*CAB operon. This operon is composed of three organized genes encoding different arabinosyl transferases that are involved in cell wall synthesis. In particular, amino acid replacement at position 306 of *emb* B has been shown in many studies to be found in EMB resistant and not in susceptible organisms. The mutation rate for EMB is $10^{-7}$with a resistance of 1 in 10^{5} bacilli (Ramaswamy and Musser, 1998).

MDR due to spontaneously occurring mutations is impossible, since there is no single gene involved in MDR and mutations resulting in resistance to various drugs arise independently. Spontaneous mutations resulting in resistance to both INH and RIF is the product of the individual probabilities i.e., 1 in 10^{14} (10^{6}x 10^{8}) (Iseman and Madsen, 1989).

It is interesting to note that mono resistance to INH is relatively common while mono resistance to RIF is quite rare. In fact, nearly 90% of RIF resistant strains are also INH resistant. Therefore, resistance to RIF may be used as a surrogate marker for MDR-TB. However, some evidence suggests that this may not be true in every setting, emphasizing the importance of collect information on the local prevalence of drug resistance patterns before implementing a molecular assay (Marinus B.).

1.1.8. Epidemiology of Drug Resistant Tuberculosis

There has been progress in the detection and treatment of MDR-TB globally. According to WHO report of 2013, 83,715 000 cases of MDR-TB were notified to WHO in 2012. In spite of the progress in the detection and treatment of MDR-TB, the number of MDR-TB cases notified represented only 28% of the estimated 300 000 cases of MDR-TB among reported TB patients with

PTB. Globally, 3.6% (2.1-5.1%) of new cases and 20.2% (13.3-27.2%) of previously treated cases are estimated to have MDR-TB. The proportions of new TB cases with MDR-TB ranged from 0% to 32.3% and the proportion of previously treated TB cases with MDR-TB at country level ranged from 0% to 65.1%. However, WHO launched Global Project on Anti-tuberculosis Drug Resistance Surveillance in 1994, Africa remain the regions where drug resistance surveillance data are most lacking, largely as a result of the scarce laboratory infrastructure (WHO, 2012).

On study conducted in Mumbai India, phenotypic DST amongst 88 isolates showed 41 (47%) sensitive, 20 (23%) monoresistant (11 INH, 2 RIF, 2 PZA and 5 ETB), 18 (20%) to be MDR and the remaining 9(10%) of polyresistant (Tolani *et al.*, 2012).

The study of anti-tuberculosis drug resistance epidemiology in 1824 Chinese population showed 1077 (59.05%) MTB isolates were sensitive to all the four first line anti-TB drugs and 747(40.95%) strains were resistant to at least one drug. Mono drug resistance was 28.73% for INH, 19.41% for RIF, 29.33% for STM, and 13.98% for EMB, respectively. MDR-TB was 16.61% with 7.63% among new cases and 33.07% among retreatment cases. Patients with previous treatment history had a more than 5-fold increased risk of MDR-TB compared with those previously not having been treated (Shao *et al.*, 2011).

A study undertaken for the first time in Mumbai reveals a high proportion of MDR-TB strains in both previously untreated (24%) and treatment failure cases (41%). Amongst new cases, resistance to 3 or 4 drug combinations including INH and RIF, was greater (20%) than resistance to INH and RIF alone (4%) (D'Souza D *et al.*, 2009).

In Egypt of the 153 (105 new and 48 retreated cases) patients enrolled for detection of RIF and INH resistant *M. tuberculosis*. Drug susceptibility testing on Bactec revealed 50 resistant cases for one or more of the first line anti-TB. 23/50 cases showed RIF resistant and 26/50 with INH resistant cases where 20/23 cases of RIF resistance were with *rpo*B mutation and 3 without mutation. 24/26 of INH resistance revealed *kat*G gene mutation and 2 cases were with wild type *kat*G (Abdelaal *et al.*, 2009).

In rural Uganda drug resistance assessment on total of 125 isolates from 167 TB suspects with a mean age 33.7 years and HIV prevalence of 67.9% (55/81) were analyzed. A majority (92.8%) of the participants were newly presenting while only 7.2% were retreatment cases. Resistance

mutations to either RIF or INH were detected in 6.4% of the total isolates. Multidrug resistance; INH and RIF resistance was 1.6%, 3.2% and 4.8%, respectively (Bazira *et al.*, 2010, 2011).

Of the 756 TB suspected patients in Cameroon, 154 (20.37%) were positive by smear microscopy. Of these, 20.77% were HIV patients. The growth of *Mycobacterium* was observed with the sputa from 149 (96.75%) subjects. All the isolates were identified as either *M. tuberculosis* or *M. africanum*. Among these, 16 (10.73%) were resistant to at least one drug (13.3% for the West region and 8.1% for the Centre). Within the two regions, the highest total resistance to one drug was obtained with INH and STM (2.68% each). MDR was observed only in the West region at a rate of 6.67%. No resistance was recorded for EMB (Assam-Assam *et al.*, 2011).

On study from Sudan MDR-TB was found in 5% of new cases and 24% of previously treated patients. Drug resistance was associated with previous treatment. Resistance was also associated with the geographic region of origin of the patient, being most frequently observed in patients from the Northern region and least in the Eastern region (Sharaf Eldin *et al.*, 2011).

From genetic profiling of *M. tuberculosis* in Tunisia drug susceptibility testing results were available for 371 isolates (98 %), of which 286 (75.5 %) were susceptible to all first-line drugs. The majority of these isolates were obtained from new TB cases (141/152; 92.7 %). The 85 remaining isolates displayed resistance to one or more first-line anti-TB drugs; 34 of these isolates (9.1 % of all isolates) were MDR strains (Namouchi *et al.*, 2008).

In Dar es Salaam Tanzania *M. tuberculosis* isolates were obtained and antimicrobial susceptibility testing was done on four antimicrobial agents namely STM, INH, EMB and RIF. A total of 280 *M. tuberculosis* isolates from 191 (68%) males and 89 (32%) female patients with no previous history of anti-TB treatment exceeding 4 weeks in the previous 12 months were tested. Fourteen (5.0%) isolates were resistant to any of the anti-TB drugs. The prevalence of primary resistance was 5.0%, 0.7%, 0.4% and 0% for INH, STM, RIF and EMB respectively. One isolate (0.4%) was MDR, with resistance to INH, STM and RIF (Urassa *et al.*, 2008).

Of 260 *M. tuberculosis* isolates from Ethiopia analyzed for gene mutations, conferring resistance to INH, RIF, or EMB were detected in 35, 15, and 8 isolates, respectively, while MDR was present in 13 of the isolates (Tessema *et al.*, 2012b).

A hospital based retrospective study used to assess the pattern of anti-TB drug resistance among previously treated TB patients referred to St.Peter's TB Specialized Hospital and among 376 culture positive for *M. tuberculosis* 102 (27.1%) were susceptible to all of the four first line anti-TB drugs; INH, RIF, EMB & STM. While 274 (72.9%) were resistant to at least one drug. Resistance to STM (67.3%) was found to be the most common and the prevalence of MDR-TB was 174 (46.3%). Trend in resistance rate among re-treatment cases showed a significant increase for any drug as well as for INH, RIF, and MDR resistance (P <0.05 for trend) (Abate *et al.*, 2012).

The DST of 37 *M. tuberculosis* isolates from Addis Ababa, 21/37 (29.8%) showed resistance to any of the drugs tested. No MDR-TB strains were observed in this study (Kassu. *et al.*, 2008).

In a study from South West Ethiopia 136 patients were enrolled from assessment of drug resistance of *M. tuberculosis* and resistance to at least one drug was identified in 18.4 %. The highest prevalence of resistance to any drug was identified against INH (13.2%) followed by STM (8.1%). There was no statistically significant difference in the proportion of any resistance by sex, age, HIV status and history of being imprisoned. The highest mono resistance was observed against INH (7.4%). MDR-TB was observed in two (1.5%) patients (Abebe *et al.*, 2012).

Among the *M. tuberculosis* strains isolated from 173 patients mycobacterial isolates from newly diagnosed pulmonary TB patients to first line anti-TB drugs in Addis Ababa showed 21.4% were resistant to at least one drug. Single drug resistance to streptomycin was observed in 16.2% , to isoniazid in 13.3% , to rifampicin in 1.2% and to ethambutol 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 a multidrug resistant (MDR) strain. However, the prevalence of resistance to more than one drug was 10.4% (Asmamaw *et al.*, 2008).

1.1.9. Diagnosis of Tuberculosis

There are a number of techniques and tests adopted for the diagnosis of Tuberculosis with their advantages and disadvantages in terms of sensitivity, specificity, cost and turnaround time (TAT), requirement of facility, training and skilled human power.

1.1.9.1. Microscopy

Despite recent advances in diagnostic microbiology, early laboratory diagnosis of tuberculosis still relies on the examination of stained smears. Microscopy of sputum smears makes a particularly important contribution since the technique is simple, inexpensive and detects those cases of pulmonary tuberculosis and MDR-TB that are most infectious. However AFB sputum smear microscopy cannot also distinguish between viable and non-viable bacilli (FMOH, 2009, IUATLD, 2007). Sputum examination by microscopy is relatively quick and easy and must be performed on all cases suspected of having tuberculosis. Most patients with infectious tuberculosis have respiratory symptoms and the use of smear microscopy in those presenting to health services with suggestive symptoms constitutes the most efficient means of case detection. Tuberculosis microscopy is also performed to assess response to treatment and to establish cure or failure at the end of treatment (WHO, 1998).

In AFB microscopy; fluorochrome, Ziehl-Neelsen, and Kinyoun staining methods can be used (Sharma *et al.*, 2005, Swaminathan *et al.*, 2010). The International Union against Tuberculosis and Lung Disease (IUATLD) and WHO recommend the Ziehl-Neelsen method under most circumstances (Rieder HL., 2007, WHO, 2009).

1.1.9.2. Nucleic Acid Amplification tests (NAAT)

Line Probe Assay (LPA) is one among NAATs which is new test that makes use of molecular technology and can identify the presence or absence of specific mutations on the genes of TB bacilli. As it is known which mutations are responsible for TB bacilli to be resistant to INH and RIF this is a rapid and accurate test to identify MDR-TB. If a patient with TB is smear positive the sputum contains enough bacilli to perform line probe assay directly on the sputum and MDR-TB can be proved on the same day or /and from culture grown isolates (FMOH, 2009).

The benefits of this test are the high degree of sensitivity (98%) and specificity (99%), the speed of the test and the potential to perform high volumes of tests per day. A drawback is that it needs well trained committed staff with a high level of quality assurance. If funds and infrastructure allow, the LPA can be used as a screening test in MDR-TB suspects, thus limiting the need for time consuming and labor intensive conventional culture and DST (FMOH, 2009).

The GenoType MTBDR*plus* detects resistance to INH and RIF simultaneously (Ling *et al.*, 2008). It is solid-phase hybridization technique in which amplified products of the resistance determining regions are allowed to hybridize to membrane bound probes covering the wild-type sequences and specific mutated sequence. The banding patterns of the hybridized oligonucleotides are visually detected after an enzyme mediated color reaction. The absence of a wild type band or the appearances of bands representing specific mutations marks the presence of drug resistance (Richter *et al.*, 2009).

1.1.9.3. Culture

Mycobacterial culture provides definitive diagnosis of TB but compared with other bacteria, multiplies extremely slowly (generation time 18-24 hours).So confirming positive and negative results by culture may take up to eight weeks. Mycobacteria also require special culture media. A variety of suitable culture media (Lowenstein Jensen (LJ)), Middlebrooks and different liquid media) are available. Quality of laboratory processing is of crucial importance. Delays in specimen transport, excessively harsh or insufficient decontamination, poor quality culture media inadequate laboratory technique or incorrect incubation temperature can adversely affect the culture yield. Specimens should also be kept at 4°C during transportation or refrigerated if delays are anticipated. False negative cultures may result from inadequate specimens, delayed transport of the specimens to the laboratory and poor laboratory techniques, or insufficient incubation period. Incubation should ideally be done for eight weeks, since some tubercle bacilli may require extended periods of incubation (FMOH, 2009).

For the detection of drug resistant TB phenotypic methods using egg or agar based LJ media are still the most utilized methods in many countries. The standard methods using LJ medium include the proportion, absolute concentration and resistant ratio method which are fairly well standardized with clinical samples, at least for the major anti-TB drugs (Canetti *et al.*, 1969, IUATLD, 2007). Among conventional methods, the proportion method is the most preferred choice. The method is based on the estimation of the proportion of mutant strains resistant to a given anti-TB drug. This is determined by comparing bacterial growth in the drug containing and the drug free control media. If the proportion of resistant bacteria is higher than 1%, the strain is considered resistant (Canetti *et al.*, 1969, Heifets, 2000). When Middlebrook 7H10/11 media is used, results are interpreted after

21 days of incubation or even earlier if there is clear growth difference between the drug containing and the drug free media (Kubica, 1985).

More recent culture method BACTEC MGIT-960 TB system, are based on liquid media. These automated methods are designed for their rapid detection of Mycobacterial growth and drug susceptibility testing of *Mycobacterium tuberculosis*. The consumption of oxygen by the growing bacterial causes the oxygen quenched fluorochrome to fluoresce under UV light. During DST, the bacterial suspension is inoculated into two MGIT tubes, one of them containing the test drug. If the anti-TB drug inhibits growth, the fluorochrome will remain quenched in the drug containing tube, while the growth control will grow uninhibited and will have increasing fluorescence. The degree of fluorescence is monitored by the instrument which automatically interprets results as susceptible or resistant (Tortoli *et al.*, 2002).

After culture species identification will be performed. There are several tests available but as a minimum, laboratories supporting drug resistant TB control programme should be able to carry out the basic biochemical tests useful for identifying *M. tuberculosis* like; niacin, catalase and nitrate or have rapid serological test kits like Cappila (FMOH, 2009).

1.1.9.4. Phenotypic Drug Susceptibility Testing

Different anti-TB drugs have different 'critical concentrations' (the breakpoint between calling a strain resistant or susceptible), which also depend on the culture medium used for DST. DST for first-line anti-TB drugs has been thoroughly studied and consensus reached on appropriate methodologies, critical drug concentrations, and reliability and reproducibility of testing. The accuracy of DST varies with the drug tested: it is most accurate for rifampicin and isoniazid and less accurate for streptomycin and ethambutol. As DST for pyrazinamide requires a different (acidic) culture medium and lacks accuracy, it is seldom performed. However the biggest drawback of phenotypic DST is the long time lag between sputum sent until a result is known. This often leads to delay of effective treatment allowing the TB patient to deteriorate, even die and increased the potential for further transmission of MDR-TB (FMOH, 2009).

1.1.10. Molecular characterization of *M. tuberculosis*

There are a number of molecular techniques which have been used successfully to characterize *M. tuberculosis* isolates. Molecular Genotyping of *M. tuberculosis* is useful for population dynamics

analysis as well as for the identification of outbreaks (van Soolingen *et al.*, 1991). Genotyping methods currently rely upon analysis of restriction profiles including Restriction Fragment Length Polymorphisms (RFLP) using IS6110 probing, amplification of selected regions of Variable Number Tandem Repeats (VNTR), Mycobacterial Interspersed Repetitive Units (MIRU), Spoligotyping and Deletion and Insertion site mapping. The genetic relationship between strains of *M. tuberculosis* can also be determined based on single nucleotide polymorphism (SNP) analysis located in intergenic spacers (Frothingham and Meeker-O'Connell, 1998, Goguet de la Salmoniere *et al.*, 2004, Gutacker *et al.*, 2006, Kamerbeek *et al.*, 1997, Mazars *et al.*, 2001, van Embden *et al.*, 1993).

1.1.10.1. Species typing and Strain typing

MTC can be rapidly analyzed by species specific deletions. Region of difference (RD) typing is among these rapid PCR based techniques used for the identification of *Mycobacterium* species. From close inspection of the flanking sequences, it is apparent that RD9 deletions occurred in MTC bacteria other than *M. tuberculosis* and *M. canettii*. Thus, RD4 and RD9-typing can be performed to differentiate between *M. tuberculosis* and other MTC members such as *M. bovis* species especially when it is important to identify sources of infection (Parsons *et al.*, 2002).

Molecular techniques are becoming essential tools for *Mycobacterium tuberculosis* complex strain typing.

IS6110 RFLP: DNA fingerprinting by using the mobile element IS6110 as a probe is considered as "a gold standard" to differentiate MTC strains (van Embden *et al.*, 1993). When used in combination with MIRU-VNTR, spoligotyping is rather a faster and more robust genotyping technique than IS6110-RFLP fingerprinting (Sola *et al.*, 2003).

Spoligotyping: Spoligotyping is a rapid PCR based method for simultaneous detection and typing of *M. tuberculosis* strains. The typing method is based on the strain dependent DNA polymorphism present at the direct repeat locus (DR) within the genome (Mostowy and Behr, 2005). The method enables classification of isolates into distinct strains, and thus allows characterization of genetic diversity of *M. tuberculosis* species in clinical samples. It has gained widespread use for studying tuberculosis outbreaks and identifying sources and chains of infection (Luciani F. *et al.*, 2008). The DR locus consists of identical 36bp DRs interspersed with 35 to 41 bp non-repetitive spacer

sequences (Groenen *et al.*, 1993). The presence or absence in the DR region of spacers of known sequence can be detected by hybridization of amplified spacer DNA to a set of immobilized oligonucleotides, representing each of the unique spacers (Kremer K., 2002).

The technique is useful not only for studies of tuberculosis epidemiology, but also for deciding about possible cross-contamination in mycobacteriology laboratories (Mazurek *et al.*, 1991).

MIRU-VNTR: Another PCR based method for the identification of various types of *M. tuberculosis* strains is MIRU-VNTR typing. This genotyping method is based on the variability in copy number of the Mycobacterial Interspersed Repetitive Units (MIRU) (Magdalena *et al.*, 1998). The discriminatory power of the 24 loci MIRU-VNTR may exceed that of the gold standard which is IS6110 RFLP method when used in combination with spoligotyping (Christianson *et al.*, 2010).

1.1.11. Treatment

Treatment for new cases of drug susceptible TB consists of a 6-month regimen of four first-line drugs: INH, RIF, EMB and PZA. In order to avoid the occurrence of drug resistance, the drugs are provided in combination of two or more (Sharma and Mohan, 2004). In countries with high TB burden, special emphasis has to be given to the treatment of new TB cases by performing drug susceptibility tests that are relevant in the identification of resistant strains (Nachega and Chaisson, 2003).

Treatment for MDR-TB is longer, and requires more expensive and toxic drugs with serious side effects. The current regimens recommended by WHO for MDR-TB lasts 20 months (WHO, 2012). The treatment of MDR-TB strategies are a subject of recurrent controversy (Caminero, 2005).

The suggested standard MDR-TB regimen in Ethiopia is 6EMB-PZA-KAN (AMK)–Lfx–Eto -Cs / 12 EMB-PZA-Lfx–Eto–Cs (FMOH, 2009).

1.1.12. Prevention and Control

Drug resistant TB threatens global TB control and is a major public health concern in several countries (WHO, 2012).

At present, tuberculosis control in Ethiopia relies on WHO recommended Stop TB Strategy and it has been implemented in the country since 2006. TB control is provided by a National Strategic

Plan is in accordance with the main strategies and focus of the WHO's Stop TB DOTS Strategy with the following five components applied to drug resistant TB:-

- Sustained political commitment
- Appropriate case-finding strategy including quality-assured culture and drug susceptibility testing
- Appropriate treatment strategies that use second-line drugs under proper case management conditions.
- Uninterrupted supply of quality-assured second-line anti-TB drugs, and
- Recording and reporting system designed for drug resistance-TB control programs that enable performance monitoring and evaluation of treatment outcomes.

Recommendations for infection control to prevent MDR-TB are essentially the same as those to prevent the spread of drug susceptible TB, with only minor differences in emphasis (FMOH, 2009).

1.2. Statement of the Problem

Emergence of drug resistant strains in tuberculosis is becoming one of the growing challenges of TB control programs. Factors such as inadequate and incomplete treatment in tuberculosis have resulted in the emergence of strains resistant to combined anti-TB drugs. Moreover, 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 in the economically most productive (15 to 49 year-old) age group (Mathema *et al.*, 2006).

According to the WHO global TB report 2012, Ethiopia is one of the 22 HBCs and there were an estimated 220,000 (258 per 100,000) incident cases of TB in 2011. According to the same report the prevalence of TB was estimated to be 200,000 (237 per 100,000). There were an estimated 15,000 deaths (18 per 100,000) due to TB, excluding HIV related deaths, in Ethiopia during the same period.

While the number of cases of MDR-TB notified in the 27 high MDR-TB burden countries is increasing and reached almost 60 000 worldwide in 2011, this is only one in five (19%) of the notified TB patients estimated to have MDR-TB indicating progress in responding to multidrug-resistant TB (MDR-TB) remains slow. Worldwide, 3.7% of new cases and 20% of previously treated cases were estimated to have MDR-TB.

Prevalence of MDR-TB in Ethiopia was estimated to be 1.6% among newly diagnosed cases and 12% among retreatment cases in 2005 (WHO, 2013).

Recent report of health facility based studies in Ethiopia showed high increase of anti-TB drug resistance with mono resistance up to 72.9% were resistant to at least one or more anti-TB drugs indicating higher prevalence of drug resistant TB for first line anti-TB drugs and MDR-TB up to 46.3% among retreatment cases (Abate *et al.*, 2012).

Economically poor and vulnerable groups are at greater risk of infection with *M. tuberculosis* compared with the general population because of overcrowded and substandard living or working conditions, poor nutrition, inter current disease (such as HIV/AIDS), and migration from (or to) higher risk communities or nations (WHO, 2005).

The current study area is one of the resource limited regions in Ethiopia where infrastructure and health facilities are very low. Benishangul Gumuz region has only two hospitals at two edges of the region; Assosa and Pawe general hospitals. Majority of the inhabitants (80%) of this region are rural with low socio economical and living standard. The region has no regional laboratory that can monitor severe health conditions like drug resistance as a whole and MDR-TB in particular.

Awi zone of Amhara regional state is geographically proximal and with almost similar settings to Benishangul Gumuz region and included to see the circulating strains and their drug resistance pattern.

1.3. Significance of the Study

Assessment of epidemiology of TB and drug resistance pattern in resource limited settings can provide basic information for regional and national health managers of the region to facilitate and adapt evidence based reliable preventive and control strategies.

It also will provide crucial information to avoid further spread of the disease and to prevent amplification of drug resistance to severe forms due to inappropriate treatment, poor case management and lack of knowledge. Early detection, treatment and establishing quality service provision system in hard to reach areas will contribute a great role for tuberculosis prevention and control programs of the country. Moreover data from the current study area will be used as baseline for further exploration.

The Epidemiology and drug resistance pattern of *M. tuberculosis* in Benishangul region and its surroundings is not still well studied and known. Therefore, the current study was designed to determine the status of the region and the outcome to be inferred for similar settings to accelerate the scaling up of case detection, case management system, introduction and adoption of new, rapid and accurate diagnostic tests which are currently promising in Ethiopia as recommended by World Health Organization.

Hypothesis

- *M. tuberculosis* strain genotypic diversity and drug resistance in Benishangul Gumuz region and its surroundings is similar with the pattern described for Ethiopia.

CHAPTER TWO: OBJECTIVES

2.1. General objective

- To determine the epidemiology and drug resistance pattern of *M. tuberculosis* in Benishangul Gumuz region and its surroundings; North West Ethiopia.

2.2. Specific objectives

- To determine the genotypic diversity of Mycobacterial strains isolated from TB patients visiting health facilities in Benishangul Gumuz and Awi Zone of Amhara region in North West Ethiopia
- To determine drug resistance pattern of *M. tuberculosis* in Benishangul Gumuz region and Awi Zone of Amhara region in North West Ethiopia
- To assess the KAP of patients towards tuberculosis in Benishangul Gumuz region and Awi Zone of Amhara region in North West Ethiopia

CHAPTER THREE: MATERIALS AND METHODS

3.1. Study Site and Period

Benishangul Gumuz Region (BGR) is one of emerging regions with limited infrastructures and health facilities and located in Northwest Ethiopia with an estimated area of 51,000 square kilometers. Its capital is located 668.7 kilometers away from Addis Ababa. The total population of the region is estimated to be 784,345 (CSA, 2007). The region has three administrative zones; Assosa, Kemashi and Metekel; Metekel is the largest zone with an area of 26,272 square kilometers followed by Assosa and Kemashi zones. The region has only two hospitals (Pawe General Hospital and Assosa General Hospital) and about 30 health centers. Awi zone of Amhara region which is geographically proximal to Benishangul Gumuz region has no hospital. The study was conducted in selected health facilities of Benishangul Gumuz region and its surroundings. These are Assosa General Hospital, Pawe General Hospital, Felegeselam health center, Gilgel Beles health center, Kemashi health center and Mambuk health centers of Benishangul Gumuz region and Chagni health center, Injibara health center and Dangila health center of Awi zone in Amhara region from April 2013- June 2014 (Figure 3.1).

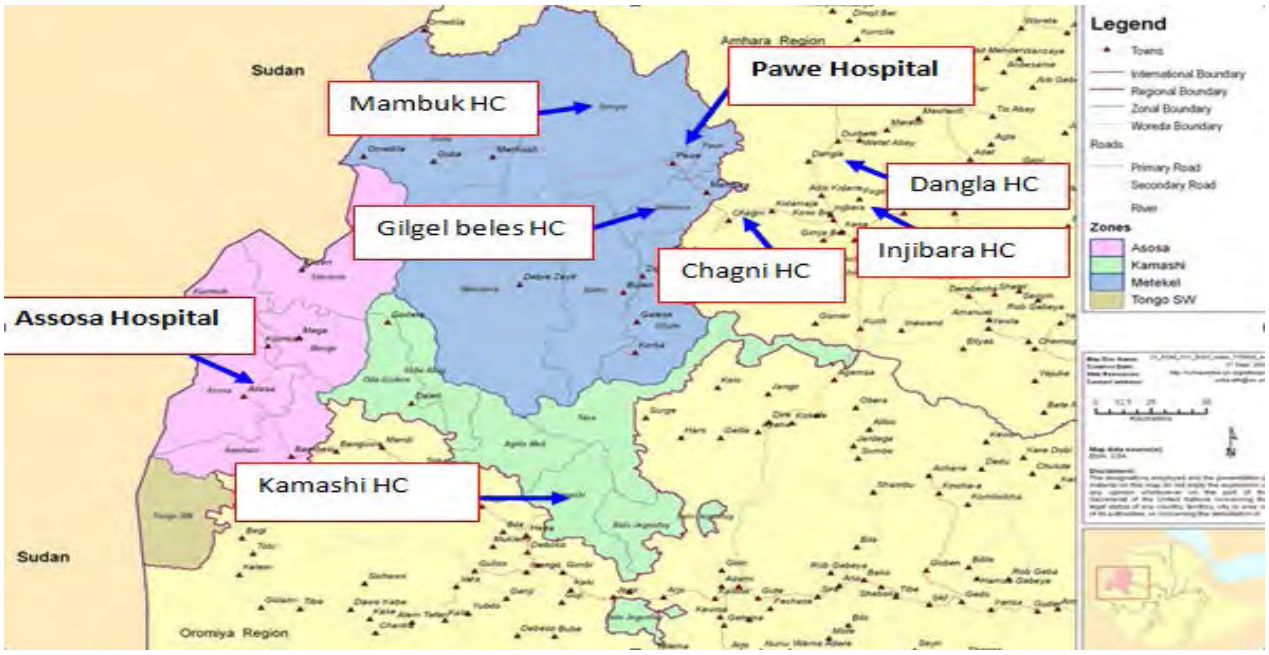

Figure 3.1: Map of Benishangul Gumuz region and its surroundings in Northwest Ethiopia

Source: www.google.images/Benishangul/gumuz

3.2. Study design

A cross sectional study was designed and conducted at health care institutions in Benishangul Gumuz region and Awi Zone of Amhara region in Northwest Ethiopia

3.3. Population

3.3.1. Source population

The source population was all patients who visited Assosa general hospital, Pawe general hospital, Gilgel beles health center, Mambuk health center, Felegeselam health center and Kemashi health center of Benishangul Gumuz region and Chagni health center, Injibara health center and Dangila health center of Awi zone, Amhara region during the study period and who were suspects for tuberculosis.

3.3.2. Study subjects

The study subjects were all newly diagnosed patients who were smear positive by Ziehl Neelson staining method from Assosa general hospital, Pawe general hospital, Gilgel beles health center, Mambuk health center, Felegeselam health center and Kemashi health center of Benishangul Gumuz region and Chagni health center, Injibara health center and Dangila health center of Awi zone, Amhara region.

3.3.3. Patient Inclusion and Exclusion Criteria

All smear positive newly diagnosed and retreatment cases were consented and included in the study for the drug resistance pattern and mycobacterial genotypic diversity study. Patients on anti-tuberculosis treatment and those unable to provide sputum were excluded.

All TB suspects were included in the knowledge, attitude and practice survey upon informed consent.

3.4. Sampling methods and procedures

Health facilities from Benishangul Gumuz region and Awi zone of Amhara region were included conveniently based on presence of both TB clinics and direct light microscopy for AFB diagnosis. Patients who visited these sites during the study period and gave their consent after being informed about the study were included in the study.

3.5. Sample Size Determination and sampling technique

For drug resistance pattern and mycobacterial diversity study non probable convenient sampling technique was employed by recruiting 112 eligible AFB positive newly diagnosed TB cases during the study period (April 2013-June 2014).

For KAP study, since the prevalence for KAP hadn't been reported for the study area, we used 50% prevalence to calculate the sample size. Therefore; the sample size calculation for KAP study using 50% prevalence at 95% confidence interval with 0.05 margins of error was 384.

3.6. Study Variables

3.6.1. Dependent Variables:

- Mycobacterial strain diversity
- Drug resistance pattern of *M. tuberculosis*

3.6.2. Independent Variables

- Previous history of anti-tuberculosis drug exposure
- Socio demographic status (age, occupation, sex, education, income, residence.....).
- KAP of patient's towards TB

3.7. Data Collection

A structured questionnaire that includes information on the socio demographic characteristics, knowledge, attitude, and practice of the respondents towards tuberculosis was used and this information was collected by assigned data collectors from TB Clinics of the study sites. Prior to the administration of the questionnaire the participants were properly informed about the study and asked consent. Laboratory information was recorded by laboratory personnel of the health facilities on laboratory information sheet prepared for this purpose.

3.8. Specimen Collection

Three consecutive sputum specimen of at least 2-5 ml volume in clean, leak-proof, with screw cap, wide-mouth, disposable containers were collected by the laboratory personnel working at TB laboratory of each health facility in the study area from patients who were suspected to have PTB during the study period. AFB microscopy was done for each sample at the health facilities. Those samples that were positive in any one of the sputum specimens were pooled and stored at -20°c freezer until transported to AHRI TB laboratory. Samples were transported in cold Ice box within a maximum of six weeks for culture. Transportation of samples was carried out by principal investigator during his frequent visits to study sites. Specimen collection and processing was done as depicted in figure 3.2.

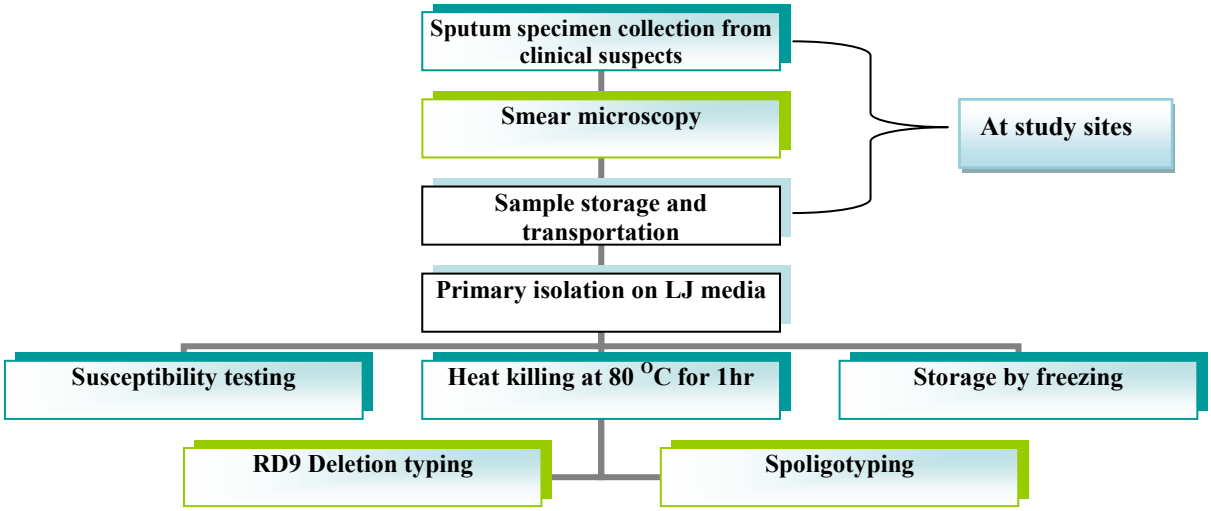

Figure 3. 2: Flow chart for data collection and laboratory works

3.9. Smear Microscopy

This is part of the routine activities of respective health facilities but it was dealt with the laboratory personnel about the sample collection. AFB Smears were prepared by taking a small portion of the purulent part (cheesy, necrotic, bloody-tinged) of sputum with an applicator stick, and smeared on a microscope slide and air dried. The standard procedure of Ziehl Neelson method was employed to confirm the presence of Acid Fast Bacilli.

Smears were examined using a light/electrical microscope by scanning up to 100 oil immersion fields before reporting a smear as negative. Acids Fast Bacilli (AFB) in specimens appeared as red

rod shaped, 1 to 10 µm long and 0.2 to 0.6 µm wide but they can also appear as coccoid or filamentous (long, slender, even branching) but the back ground and other cells stained blue.

3.10. Specimen Handling, Storage and Transportation

Smear positive sputum samples were collected by the assigned laboratory personnel working at selected health facilities and after sealing and labeling with identification code were kept at 4 ^{O}C in Ice box for transportation to AHRI core laboratory. Under certain circumstances where transportation is delayed for logistic reasons, samples were stored at -20 ^{O}C until transportation. To maintain good viability of the bacteria, stored sputum samples were transported according to standard protocol within maximum of six weeks for processing.

3.11. Specimen Processing

The smear positive sputum samples upon arrival to AHRI TB laboratory were digested and/or decontaminated by N-acetyl L-cysteine- sodium hydroxide (NALC-NaOH) method. The sputa concentrate were rechecked for smear positivity by Ziehl Neelson method upon inoculation.

3.12. Culture and Identification

Egg-based *Löwenstein-Jensen* (LJ) culture medium not more than one month of age was used for the growth of *M.tuberculosis*. Three LJ media tubes two with 0.6% glycerol and one with 0.6% sodium pyruvate were prepared for every sputum sample inoculation to maximize mycobacterial isolation. Culture tubes were incubated at 37°C and growth was observed weekly for a maximum of 8 weeks. Mycobacterial growth was confirmed by typical colony morphology and AFB staining.

Growth was checked twice a week for the first one week starting on day-3 post inoculation, then once weeks. All specimens showing growth on culture were confirmed by smear microscopy with ZN staining and were reported as " Culture positive for *Mycobacterium tuberculosis* complex pending identification". *M. tuberculosis* colonies develop well within 3 to 4 weeks as white creamy appearance on LJ media and results were reported immediately after detection and culture media kept up to 8 weeks if no growth was detected.

3.13. Drug Susceptibility Testing Methods

Drug susceptibility testing of *M. tuberculosis* was done based on indirect proportion method using Middlebrook 7H10 medium on 24 well plate (van Klingeren *et al.*, 2007). Briefly inoculum for susceptibility testing was prepared according to the McFarland standard 1.0. The amount of final drug concentration in medium for INH, RIF, STM, and EMB was 0.2, 1.0, 4.0, and 2.0 microgram (µg/ml) respectively.

A strain was considered as "susceptible" if no growth or considerably less than 1% growth is detected on the well containing the critical concentration of the corresponding drug compared to the growth control well with 1 % inoculum. A strain was considered as "resistant" if the growth on the 7H10 medium well containing the critical concentration of the corresponding drug shows more growth than the control well with the 1% inoculum. If any contamination and borderline result occurred, the tests were repeated from the colonies.

3.13.1 Template DNA for PCR and Storage

For molecular characterization and storage the genetic material from culture positive samples were prepared by heat killing. Two loops of colonies from growth were collected and mixed with 500µl of 1% TE buffer. The mixture was heated at 80^{o}C water bath for 45 minutes and sonicated for additional 15 minutes. The heat killed products were centrifuged at 13000 rpm for 10 minutes at +4^{o}C. The supernatant was used for molecular analysis and about 100µl DNA template was stored at -20^{o}C.

For *Mycobacterium tuberculosis* isolate backup, two loopful of colonies from LJ medium were mixed with 500µl of 3% Tryptone soya broth containing 20% glycerol and stored at -20^{o}C for two weeks then at -80^{o}C.

3.14. Molecular Characterization

3.14.1. RD9 Deletion typing

PCR analysis had been performed on culture positive samples to differentiate *M.tuberculosis* from other *Mycobacterium tuberculosis complex* (MTBC). Multiplex PCR on heat killed mycobacteria using RD9 primers (forward RD9_FlankFW: 5' -AACACGGTCACGTTGTCGTG-3', reverse RD9_FlankRev: 5' -CAAACCAGCAGCTGTCGTTG-3' and one internal reverse primer

RD9_InternalR: 5' -TTGCTTCCCCGGTTCGTCTG-3') were used per locus. The PCR amplification mixture used for RD9 typing was performed using a reactions in a total volume of 20µl consisting of 10 µl of HotStarTaq Master Mix (*Qiagen, United Kingdom*), 7.1µl of distH_2O, 0.3µl of each primer and 2µl DNA template from heat killed mycobacteria.

After initial denaturation at 96°C for 15 min, the PCR amplification was performed for 35 cycles, denaturation at 96°C for 30 seconds, primer annealing at 55°C for 1 minute, and elongation at 72°C for 30 seconds followed by a final extension step at 72 ^{o}C for 10 minutes. Amplicon size was determined by electrophoresis on 1.5% agarose gel which was pre-stained with 10µg/ml Ethidium Bromide. The banding patterns is visualized and photographed under an UV transilluminator. A 100bp DNA ladder was used to determine the band size of the clinical isolates.

The result was interpreted as *M. tuberculosis* (RD9 present) when a band size of 396bp was observed. The absence of RD9 with a band size of 575bp was interpreted as either *M. bovis* or *M. africanum*.

After identification by RD9 typing, spoligotyping was performed to further distinguish the circulating strains of *M. tuberculosis* (Warren *et al.*, 2006).

3.14.2. Spoligotyping

Spoligotyping was used to type *M. tuberculosis* strains based on the presence of DNA polymorphism on the direct repeat region (DR) of their chromosome. The procedure involves PCR amplification of the DR, hybridization of the Amplicons with the pre-blotted spacers and detection of hybridization by autoradiography.

Briefly 3.5 µl RNase free water, the primers (DRa 5'-GGT TTTGGGTCTGACGAC-3' and DRb 5'-CCG AGA GGGGACGGAAAC-3') 2 µl each and the 12.5 µl Qiagen Master Mix (*Qiagen, UK*) was carefully mixed in a sterile eppendorf tube. Then 20µl of the mixture of PCR mix and 5 µl of sample DNA were required for PCR reaction.

Following the initial denaturation at 98°C for 15 min, the reactions was submitted to 30 cycles of template denaturation at 96°C for 1 min, primer annealing at 55°C for 1 min, and elongation at 72°C for 30 seconds. This was followed by a final extension step at 72^{o}C for 10 min.

Primary, Secondary and Stripping spoligotyping buffers were prepared from the stock solutions and equilibrated at the required temperature (at 42°C and/or 60°C) in water bath.

Hybridization and washing with secondary buffer at 60 °C was followed by incubation with streptavidin-perioxidase conjugate in a rolling bottle at 42 °C hybridization oven. For removal of residual and unbound PCR the membrane was washed with secondary buffer at 42°C and 2X SSPE at room temperature. Finally the membrane was incubated with in Enhanced Chemiluminescence (ECL) liquid and detected by autoradiography.

PCR products were stripped from the membrane by washing in 1 % SDS at 80 °C. The stripped membrane was then be stored at +4°C in 20mM EDTA (PH = 8.0) after washed by the same buffer.

Finally, Spoligotypes in binary format were entered in Excel spreadsheet and were compared with a latest version of SpolDB4 database at http://www.pasteur-guadeloupe.fr:8081/SITVITDemo/. Spoligotype international type (SIT) numbers were used whenever pattern is found in the database and Spoligotype patterns that do not share a pattern in the updated spoligotype database were designated as ''New'' (Kamerbeek *et al.*, 1997).

3.15. Data Processing and Statistical Analysis

The data was analyzed by SPSS software version 20 (*IBM, USA*). Frequencies, proportion and summary statistics were used to describe the study population in relation to relevant variables. Odds ratio, chi-square and P-value were used to assess the presence and degree of association between drug resistance, strain difference and possible risk factors. P-value less than 0.05 were considered statistically significant.

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. Then average score was computed. Therefore those with a total score equal to or below the average were classified as having poor knowledge, while those above the average score were considered as having good knowledge. For attitude and practice section frequency table was computed and practice section was associated with knowledge level.

3.16. QUALITY CONTROL

Quality of LJ medium was assured by sterility checking and inoculating of known isolate. For Drug susceptibility testing, drug susceptible strain of *M. tuberculosis,* H37Rv, (ATCC 27294) and *M. bovis BCG* (AF 61/2122/97) were included. For RD9 deletion typing and Spoligotyping, negative control (distilled water), Positive controls (H37Rv ATCC 27294 and *M.bovis* AF2122/97) laboratory strains were run simultaneously.

Three days training was given for data collectors at study sites. AFB positive samples collected from study sites were rechecked at AHRI TB laboratory before inoculation. All procedures with positive samples were run in Biosafety Level 2 (BSL-2) cabinet and there was adherence to safe laboratory practice as per the guidelines.

3.17. ETHICAL CONSIDERATION

Before the start of work the research proposal was evaluated and approved by the Research and Ethics Committee of Department of Microbiology, Immunology and Parasitology at Addis Ababa University, School of Medicine and AHRI/ALERT ethics committee. An official letters of cooperation and support were written to the regional health bureaus explaining the purpose of the study. Respective regional and zonal health department administration wrote a letter of support and cooperation to the health facilities of the study sites.

High degree of confidentiality on participants' data was maintained and written informed consent was obtained from each study subject. The results of patients with MDR-TB were communicated to the concerned bodies for treatment and better case management as soon as possible.

3.18. DISSEMINATION OF RESULTS

The findings of this study will be presented to Department of Microbiology, Immunology and Parasitology, School of Medicine, Addis Ababa University and AHRI. The results will be communicated with the concerned bodies from study area for intervention. The findings will also be disseminated to different local, national and international organizations (Governmental and Non-Governmental) that had interest and contribution on the area of the current study. Moreover, the information will be presented in different national and international scientific seminars and workshops for dissemination and it will also be submitted to journals for possible publication.

CHAPTER FOUR: RESULTS

4.1. Socio demographic Characteristics

A total of 112 AFB positive TB patients were included for drug resistance and strain diversity study. Seventy-nine (70.5%) were male and 33(29.5%) were female with male to female ratio of 2.4:1. Their age ranged from 12-70 with a mean age of 29.58 (±10.6) years. Rural residents constituted 71 (63.4%) and 41(36.6%) of participants are urban inhabitants. Orthodox religion followers predominated with 82 (73.2%) followed by 17(15.2%) Muslim, 9(8.0%) protestant and 4(3.6%) were Catholic followers. Most of the participants 68(60.7%) were farmers, while the rest 11(9.8%) were merchants, 12(10.7%) students, 11(9.8%) government employee, and 5(4.5) daily laborers.

Fifty seven (50.9%) participants were married while unmarried, divorced and widowed comprised 47(42.0%), 4(3.6%) and 3(2.7%) respectively. Educational background of the respondents indicated 47(42.0%) illiterate, 10(8.9%) Grade 1-4, 25(22.3%) Grade 5-8, 21(18.8%) Grade 9-12 and 9(8.1%) were Diploma and above (Table 4.1).

The income of the majority 72(64.3%) respondents' is hand to mouth (<500ETB/yr) based, 71(63.4%) of them reported farming as their basic source of income.

The zonal distribution of study participants indicated that 53(47.3%) were from Metekel 29(25.9%) Assosa, 27 (24.1%) Awi and 3(2.7%) were from Kemashi. Health facility based distribution was as follows: Pawe hospital 42(37.5%), Assosa hospital 30(26.8%), Injibara health center 7 (6.3%), Dangila health center 8(7.1%), Gilgelbeles health center 5(4.5%), Kemashi Health center 2(1.8%), Mambuk health center 4(3.6%), Felegeselam health center 5(4.5%) and Chagni health center 9(8%).

Table 4.1: Socio demographics of AFB smear positive study participants in Benishangul Gumuz region and Awi zone of Amhara region, Northwest Ethiopia; April 2013-June 2014

Variables		Frequency (N)	Percent (%)	Cumulative Percent
Gender	Male	**79**	**70.5**	70.5
	Female	33	29.5	100.0
	Total	**112**	**100.0**	
Residence	Urban	41	36.6	36.6
	Rural	**71**	**63.4**	100.0
	Total	112	100.0	
Religion	Muslim	17	15.2	15.2
	Orthodox	**82**	**73.2**	88.4
	Protestant	9	8.0	96.4
	Catholic	4	3.6	100.0
	Total	**112**	**100.0**	
Occupation	Farmer	**68**	**60.7**	60.7
	Merchant	11	9.8	70.5
	Gov't employee	11	9.8	80.4
	Student	12	10.7	91.1
	Others*	4	3.6	94.6
	Daily laborer	5	4.5	99.1
	Prisoner	1	0.9	100.0
	Total	**112**	**100.0**	
Educational status	Illiterate	**47**	**42.0**	42.0
	Grade1-4	10	8.9	50.9
	Grade 5-8	25	22.3	73.2
	Grade 9-12	21	18.8	92.0
	Diploma and above	9	8.0	100.0
	Total	**112**	**100.0**	
Marital Status	Married	57	50.9	50.9
	Unmarried	**47**	**42.0**	92.9
	Divorced	4	3.6	96.4
	Widowed	4	3.6	100.0
	Total	**112**	**100.0**	

* Composed of house wives, soldiers, guards and etc...

4.1.1. Clinical presentation and previous contact history

The majority of the study participants presented with more than one sign and symptom. The predominant symptoms were cough 93(83.0%), sputum production 59 (52.7%), night sweating 56 (50.0%), fatigue /tiredness 45 (40.2%), difficulty in breathing 32 (28.6%), weight loss 46 (41.1%) and fever 47(42.0%). 55 (49.1%) complained cough and sputum production and 49 (43.8%) presented with cough and night sweating whereas 46 (41.1%) presented with night sweating and sputum production. The proportion of patients with combined clinical presentation of cough, night sweating and sputum production were 46 (41.1%). Only 35(31.25%) have had previous history of contact with known tuberculosis patient. From 79(70.5%) participants with HIV serostatus 64(80.0%) were negative while 15(18.99%) were positive. The remaining 33(29.5%) have no data on their HIV status.

4.1.2. Culture and Identification

Out of the 112 AFB positive samples processed and cultured on egg based LJ media, 3 samples were AFB negative upon concentration and became culture negative so that the remaining 109 were considered for further analysis. 89 (81.7%) were culture positive (Fig. 4.1).

Fig. 4.1: Growth characteristics of *Mycobacterium tuberculosis* on egg based LJ medium from AFB positive TB patients in North West Ethiopia, 2013/4.

All isolates from positive cultures were checked for acid fastness by using ZN staining and all became AFB positive. Deletion typing showed that all isolates were *Mycobacterium tuberculosis* (Fig. 4.2).

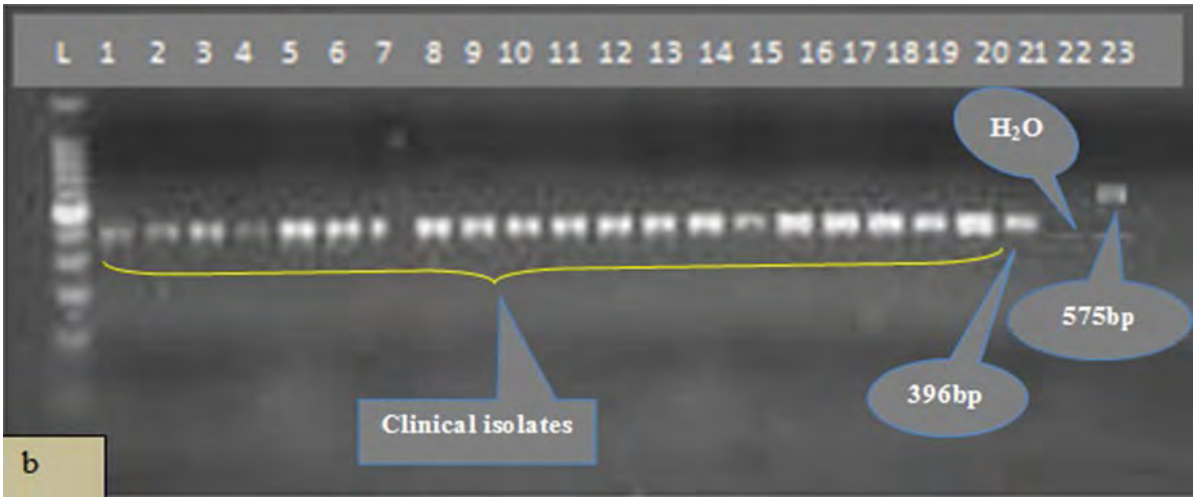

Fig. 4.2: Deletion typing indicating RD9 presence in clinical isolates. (L= 100bp ladder, Lanes1-20=clinical isolates, 21= H37Rv (positive control), 22= H_2O (negative control) and 23= *M.bovis* (positive control)

4.2. Drug susceptibility Testing

Out of 89 culture positive isolates drug susceptibility testing (DST) was not done for two isolates due to insufficient yield of colonies to make suspension for inoculation. From the remaining 87 isolates eligible for DST 14(16.5%) isolates became resistant for at least one anti-tuberculosis drug tested. Two or more drug resistance was observed in 5(5.7%) and it was always associated with INH resistance among eligible study participants. MDR was observed in 2(2.3%) amongst which one isolate was found to be resistant for all drugs tested (Table 4.2).

Over all mono resistance of 9(10.3%) was observed with 6(10.3%) INH mono resistance, 2(2.3%) ETB mono resistance and 1(1.15%) was STM monoresistance. A combined two drug resistance of INH and ETB of 3(3.5%) was observed. There was no mono resistance for RIF. Multidrug resistance of 2(2.3%) was isolated from one previously treated 1/12 (8.3%) and one new case 1/75(1.3%). The overall drug resistance was found to be 14/87(16.1%) with 2/12(16.7%) among retreatment and 12/75(16.0%) among new cases (Figure 4.3).

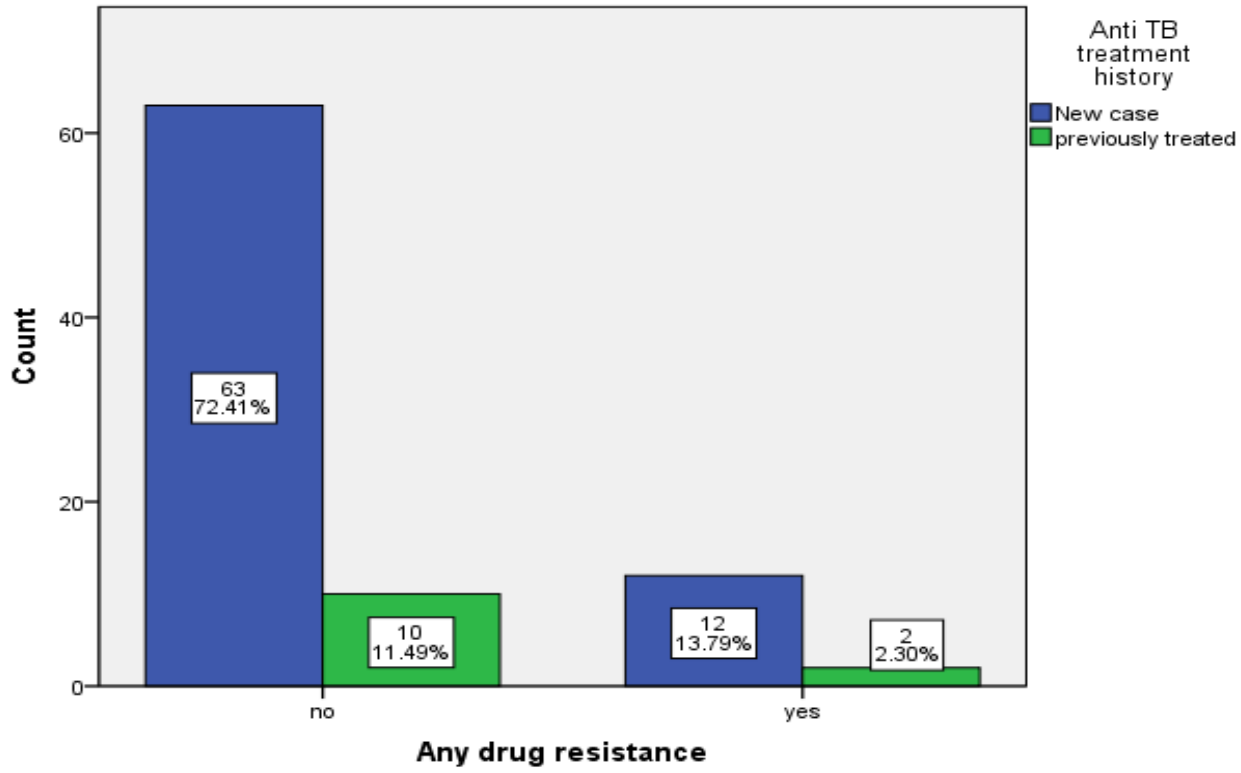

Figure 4.3: Any drug resistance distribution among new and previously treated AFB positive TB patients in Northwest Ethiopia: resource limited regions from April 2013 – June 2014.

Table 4.2: Pattern of drug resistance for four first line anti-tuberculin agents among AFB positive TB patients using Middlebrook 7H10 proportion method from Benishangul Gumuz region and Awi zone of Amhara region in Northwest Ethiopia from April 2013 -June 2014.

Drug/s tested	Susceptible N (%)	Resistance N (%)	Total N (%)
At least one drug resistance	73(83.9)	14(16.1)	87 (100.0)
INH*	76 (87.4)	11(12.6)	87 (100.0)
RIF*	85 (97.7)	2(2.3)	87 (100.0)
ETB*	80 (92.0)	7(8.0)	87 (100.0)
STM*	85(97.7)	2(2.3)	87 (100.0)
Mono drug resistance	78(89.7)	9(10.3)	87 (100.0)
INH	81(93.1)	6(6.9)	87 (100.0)
ETB	85(97.7)	2(2.3)	87 (100.0)
STM	86(98.85)	1(1.15)	87 (100.0)
Two or More drug resistance			
	82(94.3)	5(5.7)	87(100.0)
Resistance for two drugs			
INH + ETB	84 (96.5)	3(3.5)	87 (100.0)
Resistance for three drugs			
INH + ETB + RIF	86 (98.85)	**1(1.15)**	87 (100.0)
Resistance for all drugs			
INH + ETB+ RIF+ STM	86 (98.85)	**1(1.15)**	87 (100.0)
Multi drug resistance (MDR)	85(97.7)	**2(2.3)**	87(100.0)
INH + ETB + RIF	86 (98.85)	**1(1.15)**	87(100.0)
INH + ETB+ RIF+ STM	86 (98.85)	**1(1.15)**	87(100.0)

***INH**= Isoniazid ***ETB**= Ethambutol ***RIF**= Rifampicin ***STM**= Streptomycin

The proportion of isolates within any drug resistant 6(42.9%) were reported from Metekel Zone followed by 4(28.6%) isolates from Assosa zone (Table 4.3). 4/21(15.8%) isolates from Assosa, 6/38(16%) from Metekel 3/21(14.3%) from Awi and 1/3 (33.3%) from Kemashi zone were found to be resistant for at least one first line anti-TB drug tested.

Table 4.3: Zonal distribution of any drug resistance among AFB positive TB patients in Benishangul Gumuz region and Awi Zone of Amhara region from April 2013 - June 2014

			Any drug resistance		Total
			No	yes	
Zonal residence of the participant	Awi	No	18	3	21
		% within Any drug resistance	24.7%	21.4%	24.1%
	Kemashi	No	2	1	3
		% within Any drug resistance	2.7%	7.1%	3.4%
	Assosa	No	21	4	25
		% within Any drug resistance	28.8%	28.6%	28.7%
	Metekel	No	32	6	38
		% within Any drug resistance	43.8%	42.9%	43.7%
Total		**No**	**73**	**14**	**87**
		% within Any drug resistance	**100.0%**	**100.0%**	**100.0%**

4.3. Spoligotyping /Strain characterization

Spoligotyping was done as a molecular epidemiology tool used for identification and differentiation of *Mycobacterium tuberculosis* strains circulating in the area.

Table 4.4: Spoligotype pattern of Mycobacterium tuberculosis isolates from Northwest Ethiopia April 2013-June 2014.

S. No	Lab no	Spoligotype - 43 Spacers																																							Strain Classfication					
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	Octal Value	SIT	Lineage	Family	Clade	Cluster
1	104	■	■	■					■	■	■	■	■	■	■	■	■	■	■	■	■	■	■													■	■	■		■	■	■	■	■	703777740003571	289	3	CAS family	CAS1-Delhi	11
2	101	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	777777777760771	53	4	T1 Family	T	9
3	072	■	■	■	■	■	■	■	■	■					■						■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	777020377760771	New	4	X1 Family	T3-ETH	6
4	062	■	■	■	■	■	■	■	■		■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	776737777760771	New	4	T1 Family	T3	4
5	080	■	■	■	■	■	■	■	■	■											■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	777000377760771	149	4	T3 Family	T3-ETH	4
6	063	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	777737777760771	37	4	T1 Family	T3	3
7	053	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■						■	■			■	■	■	777777777720631	134	4	Haarlem3 Family	H3	3
8	050	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■						■						■	■	■	■	■	■	■	777777774020771	47	4	Haarlem1 Family	H1	2
9	007	■	■	■					■	■	■	■		■	■	■	■	■	■	■	■	■	■													■	■	■		■	■	■	■	■	703677740003571	New	3	CAS family	CAS1-Delhi	2
10	056	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■			■	■	■	■	■	■	■	■					■	■	■	■	■			777737747760760	New	4	T1 Family	T3	2
11	006	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■		■	■	■	777777777720731	49	4	Haarlem3	H3	1
12	015	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	777677777760771	498	4	T1 Family	T	1
13	019																									■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	000000007760771	4	4	Family36 Family	T1-RUS2	1
14	026	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■		■	■	■	777776777760731	336	4	X1 Family	X1	1
15	038	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■		■	■	■	776777777760731	1265	4	T1 Family	T2	1
16	043	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	717777777760771	358	4	T1 Family	T	1
17	060	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■						■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	777777037760771	174	4	H37Rv Family like	T	1
18	054	■					■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	407777777760771	411	4	T1 Family	T	1
19	051	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	717777777770771	New	4	T1 Family	Manu3	1
20	012	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■		■	■	■	777777767760731	New	4	T1 Family	T2	1
21	071	■	■	■	■	■	■	■	■		■	■	■		■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	776737377760771	New	4	T1 Family	T3	1
22	078	■	■	■	■	■	■	■	■		■	■	■		■	■	■	■	■	■						■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	776737407760771	New	4	LAM3 Family	LAM5	1
23	082									■	■	■	■	■	■	■	■	■	■	■						■			■	■	■	■	■					■	■	■	■	■	■	■	001777404760771	New	4	LAM1 Family	LAM1	1
24	089	■	■	■	■	■	■	■	■		■	■			■				■	■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	776621777760771	New	4	T1 Family	T	1
25	038														■											■	■	■	■	■	■	■					■	■	■	■	■	■	■	000020007720771	New	4	Family36 Family	T	1
26	105	■	■	■	■	■			■	■	■	■	■	■	■	■	■			■	■	■	■	■	■	■	■				■	■						■	■	■		■	■	■	763775776160731	New	4	T1 Family	T2	1
27	014	■	■	■			■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■																		■	■	■	707777740000031	New	1	EAI1 Family	CAS1-Delhi	1
28	022	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■					■	■	■	■				■	■	■		■	■	■	777737770360731	New	4	T1 Family	T2	1
29	031	■	■	■	■	■	■	■	■		■	■							■	■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	776601777760771	New	4	T1 Family	T	1
30	013	■			■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■					■	■	■	■	■					■	■	■	■	■	■	■	477777661760771	New	4	T1 Family	T	1
31	085	■	■	■					■	■		■	■	■	■	■	■	■	■	■																■	■	■	■	■	■	■	■	703377400001771	21	3	CAS family	CAS1-Kili	1
32	074		■	■					■	■	■	■	■	■	■	■	■	■	■	■	■	■	■													■	■	■		■	■	■	■	■	303777740003571	New	3	CAS family	CAS1-Delhi	1
33	025	■	■	■					■	■	■	■	■	■	■	■	■	■	■	■	■	■	■													■	■	■		■		■	■	■	703777740003531	New	3	CAS family	CAS1-Delhi	1
34	100	■	■	■					■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			■				■		■	■			■	■	■		■	■	■	■	■	703777744133571	New	3	Family33 Family	CAS1-Delhi	1
35	045	■	■	■					■	■	■	■														■										■	■	■	■	■	■	■	■	■	703600004003771	New	3	T4 Family	CAS	1
36	017	■																																				■			■	■	■	■	400000000000471	New	2	LAM7 Family	Beijing	1
37	042	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	777777777761771	1196	4	T1 Family	Manu2	1
38	023	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■				■		■	■	■	■	■	■	■	777777607762771	New	4	LAM3 Family	LAM	1
39	059	■	■	■	■	■	■	■	■			■	■	■	■	■	■	■	■				■		■	■	■	■	■					■			■	■		■	■	■	■	■	776177457413371	New	1	EAI5 Family	EAI	1

A total of 39 distinct spoligotype patterns were identified from 75 mycobacterial isolates. 46 (61.3%) isolates formed ten clusters containing 2-11 isolates (Table 4.4).

Grouping into "New" and "previously defined" showed 23/75(30.7%) and 52/75(69.3%) respectively.

The spoligotype pattern with highest number of isolate clustered was SIT_{289} with eleven isolates. SIT_{53}, SIT_{149}, SIT_{37}, SIT_{134}, and SIT_{47} showed a cluster pattern of 9, 4, 3, 3, 2 and 2 respectively. The remaining 29 (38.7%) of mycobacterial isolates represented with unique pattern i.e. not clustered.

From clustered mycobacterial isolates 6(60%) were already represented in SpolDB4.0 database while 4 (40%) clusters with 6, 4 and 2 (two clusters) isolates within each were not found at the time of analysis and designated "New" (Figure 4.4).

There is a variable clustering pattern at different health institutions. The proportion of mycobacterial isolates in the respective health institutions indicated that PHC 29(38.7%), AGH 25(33.3%), DHC 6(8%), CHC 5(6.7%), IHC 4(5.3%), KHC and FSHC 2(2.7%) each and GBHC and MHC constitute 1(1.3%). The clustering was found in only two health institutions i.e. in Pawe hospital and Assosa hospital.

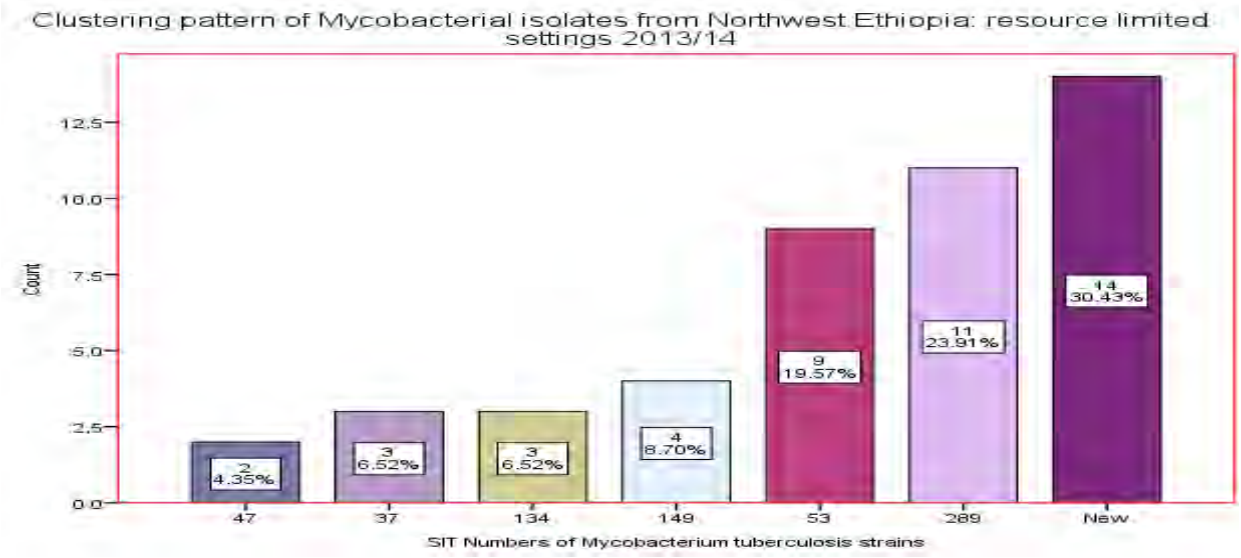

Figure 4.4: Clustering pattern of Mycobacterium tuberculosis strains from Northwest Ethiopia in April 2013- June 2014.

Lineage level classification Shared International Types of isolates indicated that majority i.e. 56 (74.7%) of the strains belong to lineage 4 (Europe, America and Africa lineage) while 17 (22.7%) of isolates belong to lineage 3 (East African and Indian lineage). Lineage 1 (Indo-Oceanic lineage) and Lineage 2 (East Asian lineage) constitutes 2 (2.7%) and 1(1.3%) strains respectively (Figure 4.5). From the total of 75 mycobacterial isolates 42 (56%) were already in the SpolDB4.0 database with distinct SIT number while 33 (44%) were not found in data base and assigned peculiar SIT number. From 42 strains found in data base at time of analysis 31(73.8%) were assigned as Lineage 4 with predominant strains SIT_{53} (n=10, 32.3%), SIT_{149} (n=4, 13%), SIT_{37} (n=3, 9.7%) and SIT_{47} (n=2, 6.5%). The remaining 11(26.2%) strains from database were assigned as lineage 3 with dominant strains of SIT_{289} (n= 8, 72.7%) and SIT_{298} (n= 2, 18.2%). In another hand lineage 2 and lineage 1 contained one (1.3%) "New" strain each with SIT number was not assigned in database.

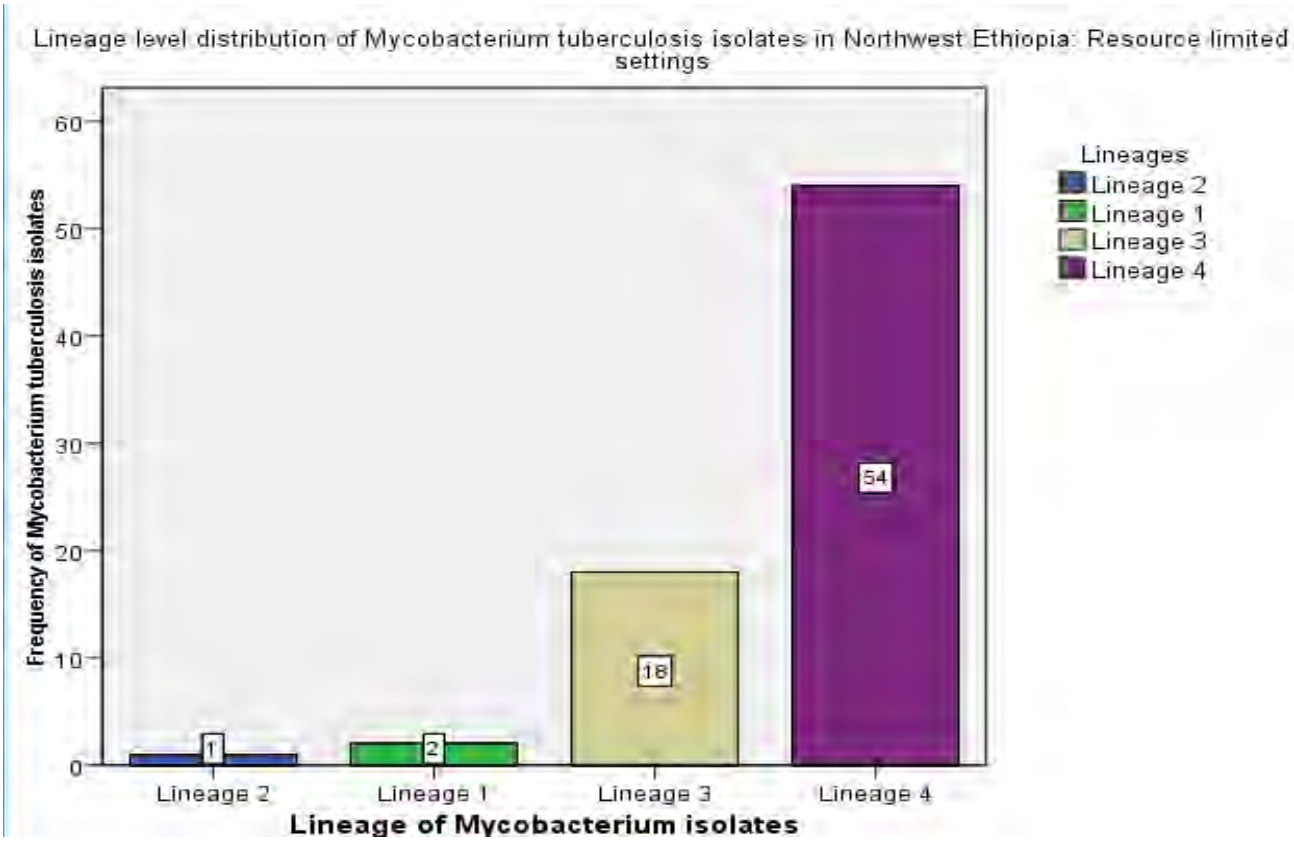

Figure 4.5: Lineage level distribution of Mycobacterium tuberculosis strains in Northwest Ethiopia: resource limited region in April 2013- June 2014.

Zonal distribution of *Mycobacterium tuberculosis* strains showed that "New" strains are predominant in all zones. From previously described strains in database. SIT289 took the lead in Assosa and Awi zones where as SIT53 predominates in Metekel zone (Table 4.5).

Table 4.5 : Distribution of internationally shared types of Mycobacterium tuberculosis in zonal administrations of Northwest Ethiopia: resource limited region from April 2013-June 2014.

Metekel Zone			Assosa Zone			Awi Zone			Kemashi Zone		
Strains (SITs)	**N**	**%**	**Strains (SITs)**	**N**	**%**	**Strains (SITs)**	**N**	**%**	**Strains (SITs)**	**N**	**%**
New*	12	16%	**New**	14	18.7%	**New**	5	6.7%	**New**	2	2.7%
53	7	9.3%	**289**	4	5.3%	**289**	4	5.3%	**149**	1	1.3%
289	3	4%	**37**	2	2.7%	**47**	2	2.7%			
498	1	1.3%	**53**	1	1.3%	**134**	2	2.7%			
49	1	1.3%	**336**	1	1.3%	**53**	1	1.3%			
4	1	1.3%	**174**	1	1.3%	**411**	1	1.3%			
37	1	1.3%	**149**	1	1.3%	**21**	1	1.3%			
358	1	1.3%	**134**	1	1.3%	**149**	1	1.3%			
149	1	1.3%									
1265	1	1.3%									
1196	1	1.3%									

*Indicating database status. It doesn't mean all new are of a single spoligotype pattern (*see Table 4.4*)

4.4. KAP of TB suspects towards tuberculosis

4.4.1 General description of participants and clinical presentations

A total of 383 eligible respondents were included for KAP study. Of these, 231(60.3%) were male and 152(39.7%) were female. The participants' age ranged from 11-89 years with median age of 33.34 (±14.10) years. The majority 242(63.2%) were rural residents and 221(55.1%) used farming as a means of income generation. 165(43.1%) were illiterate and had never attended formal education. Most of the respondents 260(67.9% were Orthodox religion followers.

The participants presented to health facilities with multiple symptoms. Cough for more than 2 weeks in 274(71.5%), Sputum production 117(30.5%), night sweating 144(37.6%), fatigue 130(33.9%), difficulty in breathing 82(21.4%), weight loss 96(25.1%) and fever in 97(25.3%). 56(14.6%) presented with all the above mentioned clinical symptoms.

4.4.2 Participants' basic knowledge about tuberculosis and their source of information

The respondents' sources of information about tuberculosis were: health professionals in 131(34.2%), radio 100(26.1%), television 42(11.0%), from community 39(10.2%) and 23(6%) were from family members while 20(5.2%) had never heard about tuberculosis. The remaining 28 (7.3%) got the information from different sources such as magazine, neighbor, fliers, posters, religious leaders and teachers. Tuberculosis symptom related knowledge assessment indicated that 87(22.7%) mentioned cough less than 2 weeks as symptom of TB whereas 207(54%) said cough more than 2 weeks, chest pain 92(24%), shortness of breath 74(19.3%), fatigue 101(26.4%), weight loss 68(17.8%), headache 33(8.6%) and fever 52(13.6%). The overall average of TB symptom related knowledge was poor for 337(88%) participants. Previous history of treatment has statistically significant association with participants average knowledge about TB symptoms (p= 0.007).

The majority of the respondents, 254(66.3%) knew that TB transmission is through aerosol route with 236(61.6%) participants' good average knowledge about TB transmission while 81(21.1%) do not know about it. Occupation has statistically significant association with average knowledge of participants about transmission of TB (p= 0.029). Similarly 223(58.2%) responded TB transmission can be prevented by covering mouth and nose while coughing and sneezing, while125 (32.6%) do not know and overall 163(42.6%) participants had poor knowledge about it. Participants average

knowledge about TB prevention is found to have statistically significant association with occupation (p=0.052).

Although 315(82.2%) knew that everybody is at risk of acquiring TB 22(5.7%) and 11(2.3%) believed that only poor and PLWH are at risk respectively. Majority 368(96.1%) believed that TB is curable. In regards to treatment of tuberculosis 355(92.7%) responded anti-TB drugs are used while 15(3.9%) believed that tuberculosis is treated by using medicinal plants and 10(2.6%) didn't know. 294(76.8%) knew that the treatment for tuberculosis free while the rest 89(23.2%) did not know.

4.4.3 Participants general attitude and practice towards tuberculosis

317(82.8%) of the respondents feel that TB is a very serious and severe disease. 222(58%) do not want to get more information about the disease while the majority, 361(94.3%) want to get more information about the disease Only 11(2.9%) of respondents said that they can talk freely if they have tuberculosis and 290(75.7%) will talk only with health personnel, 58(15.1%) with family members and 10(2.6%) do not want anyone to know. 225(58.7%) feel that they might acquire the disease and their reaction to being infected is fear 148(38.6%), 124(32.4%) indifferent, 54(14.1%) sorrow and 13(3.3%) feel happy to know their status.

Seventy six (19.8%) of the participants were worried about long treatment, 11(2.9%) stigmatization and discrimination, 38(10%) fear side effects, 37(9.7%) transmission to family members and others, 34(8.9%) death and 24(6.3%) curability associated with tuberculosis. The rest 109(28.5%) were not seriously worried.

In response to health service seeking behavior, 232(60.6%) visited health institution twice and more per year, 39(10.2%) once a year, 7(1.8%) twice in last five years, 6(1.6%) never visited in the last five years and 78(20.4%) would like to visit whenever they feel sick.

Reasons given for delay in health seeking behavior was related to cost and affordability in 63(18.5%), distance and /or lack of transportation in 56(16.4%), 27(8.1%) don't know where to go, 12(3.5%) lacked confidence on health personnel and 14(3.7%) mentioned different factors while 169(49.6%) stated that they don't know the reason for the delay. There was no response from 42(11%) participants.

Univariate analysis showed that previous history of treatment is significantly associated with average knowledge about symptoms of TB (P =0.012, odds ratio 5.556; CI=1.461-21.126). Average knowledge about transmission with profession being student has statistically significant association (P=0.015; Odds ratio 17; CI= 1.752 -165). Outcome variables (any drug resistance, multidrug resistance, average knowledge about TB prevention) were analyzed for any association with age, sex, residence, occupation, educational level, income status, previous history of treatment contact history and HIV status of the participants using Univariate and multiple logistic regression and showed no significant association (P> 0.05).

CHAPTER FIVE: DISCUSSION

Mycobacteria genetic diversity studies are very crucial in order to identify *M.tuberculosis* strains circulating in different geographic areas and to follow their transmission dynamics. Different mycobacteria strains have difference in their pathogenicity, rate of mutation and thereby degree of drug resistance. Therefore, identifying the drug resistance profile of circulating strains in particular region is important evidence that can be used to develop new and improved intervention strategies to prevent and control the transmission of tuberculosis.

Little is known about *Mycobacterium tuberculosis* strain diversity, drug resistance pattern and Knowledge, Attitude and Practice (KAP) of TB suspects towards tuberculosis in Benishangul Gumuz region and Awi zone of Amhara region in Northwest Ethiopia.

We therefore have conducted this study in Benishangul Gumuz region and Awi zone of Amhara region in Northwest Ethiopia to contribute in filling the gap.

Our study has revealed that one or more drugs resistance rate of 16.5% which is relatively higher than the reports from studies conducted in different parts of the country 15.6% (Demissie *et al.*, 1997) and 15.8 % (Tessema *et al.*, 2012 a). The relatively higher rate of one or more anti-tuberculosis drug resistance could be due to multifactorial reasons including unintended use of drugs, illegal distribution of anti-tuberculosis drugs by individuals and private vendors, poor compliance of patients to anti-tuberculosis drugs and genetic diversity of circulating strains.

In the current study, a high rate of resistance to Isoniazid (6.9%) was observed. Our finding is relatively comparable to a resistance rate of 8.4% and 9.5% (Demissie *et al.*, 1997; Seyoum *et al.*, 2014). In contrary, some other studies have reported a lower resistance rate compared to that of us (Kassu *et al.*, 2008; Yimer *et al.*, 2012; Tessema *et al.*, 2012a; Bruchfeld *et al.*, 2002; Esmael *et al.*,2014a). The higher rate of isoniazid monoresistance observed could be due to the presence of different strains in different geographic areas, isoniazid preventive therapy that makes the drug to be easily accessible and patient's poor compliance due to lack of knowledge.

In our study Ethambutol monoresistance of 2/87(2.3%) observed is in agreement with the finding reported by Kassu. *et al.*,(2008). However, our Ethambutol monoresistance finding is relatively higher than the findings reported by others (Seyoum *et al.*, 2014; Esmael *et al.*, 2014a; Bruchfeld *et*

al., 2002 ; Demissie *et a l.*, 1997 ; Yimer *et a l.*, 2012 and Tessema *et a l.*, 2012a). T his hi gher resistance rate o f E thambutol m onoresistance i n t he cu rrent s tudy area could b e d ue t o genetic difference in strains.

Streptomycin monoresistance rate of 1(1.15%) is comparable with finding of 1.5% by Tessema *et al.* (2012a) but is lower than Streptomycin monoresistance rates reported by others (Kassu. *et al.*, 2008, Yimer *et al.*, 2012, A smamaw *et al.*, 2008, Hussein *et al.*, 2013 and Esmael *et al.*, 2014a). The l ower r ate of S treptomycin m onoresistance in our s tudy area could be due t o di fference i n strains c irculating a nd i ts in clusion a s th e tr eatment r egimen of r etreatment cas es d uring t he intensive phase only which makes it to be less accessible.

Our study showed that Rifampicin monoresistance rate of 0% which is similar with that of reported by Yimer *et a l.*,(2012). H owever, others s tudies r eported s lightly higher but c omparable rate o f Rifampicin mo noresistance (Esmael *et a l.*, 201 4a, B ruchfeld *et a l.*, 2002, K assu. *et a l.*, 2008, Seyoum *et al.*, 2014). The lower Rifampicin resistance rate observed in all studies might be because of its inclusion in anti-TB treatment regimen is relatively recent.

Overall, multidrug resistance of 2/87(2.3%) was observed in the current study. The rate of MDR among new and retreatment cases was 1(1.3%) and 1(8.3%) respectively. The rate of MDR found in our study is higher than the rates of MDR reported by others (Kassu. *et al.*, 2008, Seyoum *et al.*, 2014, Asmamaw *et al.*, 2008, Demissie *et al.*, 1997 and Bruchfeld *et al.*, 2002). The higher rate of MDR in the current study could be due geographical and genetic difference in the circulating strains and due to variation in the rate of mutations in different settings. In addition to this, due to limited health service ac cess, an ti-tuberculosis dr ugs can a lso be pr ovided b y p rivate ve ndors t hat c ould result in the development of multidrug resistant *Mycobacterium* strains due to inappropriate dosing and interruption in frequency.

In our study 1/87(1.15%) isolate was found to be resistant for all drugs tested and showed mutation for *rpo*B a nd *katG* genes w hen confirmed us ing l ine pr obe a ssay. A previous study had al so reported mycobacterial strains resistance to all first line anti-tuberculosis drugs of 2.8% (Tessema *et al.*, 2012a). This c ould show the pos sible e xistence of f avorable environment for de velopment o f XDR T B w hich results in m ore e conomical, s ocial a nd ps ychological c risis f or t he pa tients a nd

country in general and thereby studies of this kind and/or establishing continuous sentinel surveillance for drug resistance tuberculosis is essential.

From 75 *Mycobacterium tuberculosis* isolates typed using spoligotype 43(57.3%) were already present with assigned international shared type numbers in SITIVIT database. 32(42.7%) isolates typed were not included in the database and apportioned as "New".

The proportion of "New" strains in this study is slightly higher than studies conducted in different parts of the country (Esmael *et al.*, 2014b, Garedew *et al.*, 2013, Diriba *et al.*, 2013) .This difference could be due to circulation of different strains in different geographic areas (Ford *et al.*, 2013).

In the current study 39 particular spoligotype patterns were observed. The spoligotype pattern observed for the 39/75 (52%) of the isolates indicates high level of mycobacterial diversity with difference in different level of clustering pattern. The predominant strains among previously defined database with international shared types were SIT289 and SIT53 which constituted 25% and 20.5%of the database respectively. This proves that these two strains are the dominant strains circulating in the present study region.

Clustering pattern in previously defined isolates and 'New' strains showed a slight difference. The prior showed 32 isolates clustered into 6 groups of 2-11 strains in each and 10 showed single spoligotype pattern (singleton). From 43 isolates assigned as "New" 14 isolates found in a groups of four containing 2-6 isolates in each cluster and the remaining 19 represented by a single pattern. In another study 38/46 isolates previously described in database showed 7 spoligotype patterns (Agonafir *et al.*, 2010) . This finding indicated high diversity of mycobacterial isolates and still ongoing transmission in the community from both previously defined strains and newly identified ones.

Relatively higher rate of clustering was seen in 46/75 (61.3%) isolates which is an indicator of ongoing transmission despite scattered settlement of population in the region the value indicated is very high.

In the current study SIT289 is the predominant strain circulating in the region with about 11/75 (14.7%) proportion out of the total strains identified. These strains were never reported as

predominant in none of Ethiopian studies and neighboring Sudan (Sharaf Eldin *et al.*, 2011) suggesting that geographic and demographic preference of isolates. This will urge a need for further steps to address all facets so as to have a real national picture about circulating mycobacterial strains in the country.

Other strains prevalent in the study area are SIT53, SIT149, SIT37 and SIT134. These strains were also reported as prevalent strains in different parts of the country from Eastern Amhara, Debrebirhan Hospital, Addis Ababa, Southwest Ethiopia, Fitche and Butajera (Esmael *et al.*, 2014b, Garedew *et al.*, 2013, Agonafir *et al.*, 2010, Deribew *et al.*, 2012, Firdessa *et al.*, 2013). The possible explanation for these strains in our study area could be the presence of highly diversified population and ethnic groups resettled from different parts of the country during the Derge regime.

One isolate was identified as Beijing clade by using *SpolDB4.0* and assigned in lineage two according to classification of clades into lineage (Brudey *et al.*, 2006). This strain and previously unreported 32(42.7%) isolates found in clustering 2-6 a group needs further analysis using a tool with high degree of discriminatory power (Firdessa *et al.*, 2013).

Assigning family level classification majority strains identified were CAS1-Delhi, T, T3, T3-ETH, H3, T2, LAM1 and H1 with cumulative of 86.6% and proportion of 22.7%, 21.3%, 13.3%, 13.3%, 5.3%, 5.3%, 2.7%, 2.7% respectively. Similar studies from northwest Ethiopia and Sudan also indicated CAS-Delhi strains as predominant circulating strains (Tessema *et al.*, 2013; Sharaf Eldin *et al.*, 2011). Unlike the current study in most of the studies in Ethiopia T family is predominant (Diriba *et al.*, 2013, Firdessa *et al.*, 2013, Deribew *et al.*, 2012, Agonafir *et al.*, 2010 and Esmael *et al.*, 2014b). The difference in circulating strains in different parts of the country may suggest adaptation of isolates to different population and geographic settings.

In Sudan genotypic study also indicated CAS1-Delhi to be highly predominant clade and high clustering in the current study suggesting its cross border active transmission (Sharaf Eldin *et al.*, 2011).

By assigning the spoligotype patterns in to lineages showed lineage 4 to be the predominant lineage followed by lineage 3 which are modern lineages. This finding is comparable with former studies in Ethiopia that found families of the modern lineages dominating and associated with recently transmitting strains in the country (Agonafir *et al.*, 2010 and Mihret *et al.*, 2013). The presence of lineage 1 (ancestral lineage) in the study area which is believed to be the ancient lineages may indicate importance of further phylogenic study in border areas of Northwest Ethiopia for the interest of *Mycobacterium tuberculosis* evolution.

Although prevalence of 2.3% MDR TB cases in this study seems lower, there are favorable conditions of high Isoniazid monoresistance and strains in families like Haarlem, Beijing and EAI which are believed to be associated with multi drug resistance due to their higher mutation rate (Ford *et al.*, 2013).

The absence of Lineage 7 (Woldeya lineage) whereas collections from the eastern part are known to include it and the presence of lineage 1 (ancestral lineage) & Beijing strain which are rare in Ethiopia may suggest the importance of further phylogenic study in border areas of Northwest Ethiopia for the interest of *Mycobacterium tuberculosis* evolution.

In this study there was no statistically significant association between MDR, strains identified, HIV status of participants and socio-demographic characteristics. This could be due to low number of study subjects and needs further wide ranged study at community level.

KAP data provides national TB programme managers and their staff with the fundamental information needed to make strategic decisions (WHO, 2008). In this study health professionals 131(34.2%) followed by public radio 100 (26.1%) were most frequently reported sources of tuberculosis information. Similar sources of information about TB were also reported with comparable findings (Mesfin *et al.*, 2005; Melaku *et al.*, 2013). This could be due most of rural community have better access for radio and health worker than other source of information.

Our finding showed that nearly one quarter (21.1%) of participants do not know about TB transmission. Similar findings supporting the current result were reported in different parts of the country (Esmael *et al.*, 2013; Mesfin *et al.*, 2005). These finding is one of the indicators that could pause prevention and control efforts and thereby sustain the chain of TB transmission in the community.

Though more than half (58.2%) of participants responded that TB transmission can be prevented by covering mouth and nose while coughing and sneezing, still there are about one-third 125(32.6%) of respondents that do not know about TB prevention with overall 163(42.6%) participants with poor knowledge about it which is found to have statistically significant association with occupation of being farmer (p=0.052). Similar study elsewhere in the country revealed that 30.4% participants do not know about prevention and control of TB (Abebe *et al.*, 2011). The wider gap seen in this regard could urge the need for additional tools to be put in place to halt the continuing spread of TB burden in the community through provision of health education.

In the current study the overall average TB symptom related knowledge of respondents was poor among 337 (88%) participants. Similarly lower level of TB symptom related knowledge was reported from other studies (Abebe *et al.*, 2011; Bati *et al.*, 2013; Esmael *et al.*, 2013).

Although the majority (>80%) of respondents knew that everybody is at risk of acquiring TB and believed that it is curable by taking anti-TB drugs from health institutions, 89/383(23.2%) did not know that anti-tuberculosis drugs are given freely at no cost to the patient. This finding is concurrent with the finding from other studies (Esmael *et al.*, 2013). This finding showed the need for comprehensive health education including about the available health services.

High level of fear of death, stigmatization and discrimination, hardly accepting being TB patient and poor interest to talk about TB is associated with attitudinal misconception.

Health service seeking behavior of respondents was observed in 153/383(40%) of participants who visited health institution once and/or less per year because of cost and affordability (18.5%), distance and/or lack of transportation (16.4%) and other factors (15.3%) while 49.6% don't know the reason for the delay is supported with findings from another study (Gele *et al.*, 2009). As the result of scattered settlement of population in the current geographical areas coverage by health institutions and accessibility is limited. Delay was observed in healthcare seeking behavior, diagnosis and treatment which could be due to lack of knowledge and this attributes to further aggravation of the transmission rate and morbidity/mortality caused by tuberculosis.

CONCLUSION

The current study is the first in its kind for the region and addressed the issue of *Mycobacterium tuberculosis* drug resistance pattern, *Mycobacterium tuberculosis* strain diversity and assessed KAP of TB suspects residing in Benishangul Gumuz region and Awi zone of Amhara region about tuberculosis.

Relatively a higher rate (16.5%) of resistance to one or more drugs was observed with higher rate of monoresistance to isoniazid (6.9%). Multidrug resistance of 2/87(2.3%) was observed with 1/75(1.3%) and 1/12(8.3%) among new and retreatment cases respectively. Strain resistant to all first line anti-TB drugs tested and isolated from new case was SIT1196 of T1 family and clade Manu2. This strain is not common in other parts of the country and to be found in a MDR-TB strain may need special attention for further investigation.

There was no statistically significant association observed between drug resistance and HIV status, previous history of treatment, age, contact history, residence and strain types.

In the current study high rate of *Mycobacterium tuberculosis* strain diversity with a high proportion of "New" isolates 32(42.7%) was observed. From a total of 39 particular spoligotype patterns, 10 patterns were observed with different level of clustering ranging from 2-11 isolates in each cluster. The remaining 29/75(38.7%) isolates were found as a single spoligotype pattern (singleton).

SIT289, SIT53, SIT149, SIT37 and SIT134 were the predominant strains circulating in the study region with a proportion of 14.7%, 12.0%, 8.0%, 5.3% and 4.0% respectively. Lineage 4 and lineage 3 were found to be principal lineages identified in the current study area.

Mycobacterium tuberculosis families of T3-ETH, H3, T2, LAM1 and H1 with strains identified CAS1-Delhi, T and T3 were identified with a cumulative percentage of 86.6% and with a proportion of 22.7%, 21.3%, 13.3%, 13.3%, 5.3%, 5.3%, 2.7%, 2.7% respectively were observed.

Lineage level assignment showed that lineage 4 (Euro-American lineage) to be the predominant lineage in the current study area. This could be due to the long existence of Tana Beles / Salini Construttori project which was led by Italians in connection with resettlement programs in the region. Even though MDR was found to be 2.3% only, existence of mycobacterial strains in families like Haarlem, Beijing and EAI which are believed to be associated with multi drug

resistance due to their higher mutation rate (Ford *et al.*, 2013) and higher rate of Isoniazid monoresistance observed are bench mark indicators for further increment of MDR TB cases in the area.

KAP survey among TB suspects indicated the gap in terms of knowledge about symptoms, mode of transmission and means of prevention.

The results of this study revealed a low level of knowledge about main symptom of TB, mode of transmission and means of prevention. In addition, the study showed unfavorable attitude and poor practices towards TB. Moreover, substantial numbers of participants were not aware of the free service available. Health seeking is delayed due to cost and unaffordability, lack of transport and or distance from health care institutions.

RECOMMENDATIONS

Based on our findings the following recommendations are provided:

- The existence of MDR and different level of resistances may need to have continuous drug resistance surveillance and Drug Susceptibility Testing facilities for all eligible TB patients at least at regional level.
- Higher mycobacterial strain diversity and relatively higher number of "New" strains observed in the current study area would urge the need for more elaborative study in mycobacterial strain evolution and molecular epidemiology in the study area.
- The gap seen on knowledge, attitude and practice towards tuberculosis can be minimized by having institutional and community based disease specific health education on top of efforts already being made in remote regions.
- Strong multi-sectorial collaborative effort and political commitment will have crucial role in the control of only prescribed distribution and use of anti-tuberculosis drugs so as to prevent drug resistance development.

MERIT FINDINGS OF THIS RESEARCH

- Since it is the first in kind in the study area; it will provide evidence based new data for the scientific community and national and international policy makers to design appropriate strategies of intervention.
- MDR TB Cases were detected and treated in the study which has contributed directly in the management of the patients and in control of potential sources of infection with resistant strains in the community.

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ANNEX I

PARTICIPANT INFORMATION SHEET

Addis Ababa University School of Graduate Studies

Armauer Hansen Research Institute (AHRI)

Name of the Principal Investigator: Tekle Airgecho

Name of the organization: Addis Ababa University Medical Faculty

Name of the sponsor/s: School of Graduate studies/AAU and AHRI

This information sheet is prepared with the aim of studying Epidemiology and drug resistance pattern of *Mycobacterium tuberculosis* in Northwest Ethiopia: resource limited settings.

Duration: Study will take 6 months or more

Purpose:

The purpose of this research is to study the **Epidemiology and drug resistance pattern of *Mycobacterium tuberculosis* in Northwest Ethiopia: resource limited settings.**

It is has been known that tuberculosis is one of the killer and preventable disease. Information on the Epidemiology and pattern of drug resistance to tuberculosis will have significant importance for the efforts being made for the control and prevention of tuberculosis in resource limited settings.

Different studies suggest that the emergence of drug resistant M. tuberculosis strains pose a major challenge to TB prevention and control efforts. Even though much has been done still incidence of drug resistant tuberculosis is increasing worldwide. The condition is fueled by socioeconomic factors and chronic diseases like HIV/AIDS. So knowing the epidemiology and drug resistance pattern of tuberculosis in resource limited settings will help to develop preventive and control measures and to adopt for rapid detection and treatment of cases. Moreover it will help for better management of cases.

Procedure:

If you are willing to participate in this research, you need to understand and sign the agreement form.

Upon your agreement you will be examined by health professional of the Hospital/Health Center to check your general clinical conditions. Based on this examination, the health professional will decide whether to participate on this research or not. If you are not be able to participate in the study by the recommendation of the health professional or based on your own interest, it has no effect in any way in obtaining the regular health services delivered at the Hospital/Health center.

We will ask you to provide three consecutive sputum samples, (Spot- Morning- Spot). Laboratory examination will be done on the samples with no extra cost from you. In addition to this we will ask you some questions regarding your background, knowledge and awareness about tuberculosis. All the clinical and laboratory examination results will be kept confidential by coding system whereby no one except the health professional and principal investigator will have access to your clinical and laboratory results.

Risk and Discomfort

By participating in this research project, you will not feel discomfort and it is part of routine TB care service. However, when we ask you some related questions, you may be bored and spend sometimes with us which may potentially affect your social (work) time.

Benefits

Your participation in this research project may not have direct benefit but depending on your laboratory result there will be arrangement for treatment and management. In addition, your participation is likely to help us in understanding the epidemiology and pattern of anti-TB drug resistance of Mycobacterial isolates and M. tuberculosis strain types in population and may benefit in the future to design prevention, control and early treatment strategy of this disease in our society. If you are also identified with MDR-TB, you will be referred to appropriate health institution for further treatment and care.

Incentives

You will be compensated for the time spent during interview. However, there will be arrangements for treatment depending on the outcome of the study result.

Confidentiality:

Any identifiable data about you from this research will be kept confidential. It will be stored in a file, which will not have your name on it, but a code number assigned to it. Which number belongs to which name will be kept under lock and key, and it will not be revealed to anyone except the principal investigator and health professional involved in your treatment.

Participant right

You have full right to refuse from participating in this research and this will not affect your treatment or health services you get at this health institution in any way. You also have full right to withdraw from this research at any time you wish to, without losing any of your rights as a patient in your health institution.

Contact persons

If you have any questions about this research project, or need further clarifications feel free to contact the following individuals at any time.

Principal Investigator: Tekle Airgecho (MSc Candidate at AAU)

Mobile: 091-175-69-79

Email: teklemicro@gmail.com

Supervisor: Demissew Beyene

Mobile: 091-140-20-60

Email: beyene88@gmail.com

You can also call the ethics committee if you have any questions concerning your rights as a participant:-

AHRI/ALERT Ethics Review Committee: Tel: 0113-481289

ANNEX II

የጥናቱ ተሳታፊዎች የመረጃ ቅጽ

አዲስ አበባ ዩኒቨርሲቲ የድህረ ምረቃ ትምህርት

የአርማወር ሃንሰን ምርምር ተቋም

የተመራማሪዉ ስም፡ ተክሌ ኤርጊቾ

የድርጅቱ ስም፡ አአዩ፤ ህክምና ፋኩሊቲ፤ማይክሮባዮሎጂ፣ኢሚዉኖሎጂና ፓራሲቶሎጂ ት/ክፍል

የጥናቱ ስፖንሰር ስም፡ አርማወር ሃንሰን የምርምር ተቋም

በሰሜን ምዕራብ ኢትዮጵያ የቲቢ በሽታ አምጪ ባክቴሪያ መድሃኒት የመላመድ ሁኔታና የባክቴሪያዉን ዝርያ አስመልክቶ ለሚደረግ የምርምር ጥናት ለተሳታፊዎች የተዘጋጀ መግለጫ፡፡

<u>የጥናቱ ዓላማ፡-</u>

የቲቢ በሽq ማይኮባክቴሪያ በተባሉ ባክቴሪያ የሚመጣ ተላላፊ በሽq ሲሆን የ^ነዚህ ባክቴሪያ የመተላለፍና መድሀኒትን የመላመድ ሁኔታ እንደየዝርያቸው ይለያያል፡፡

የዚህ ጥናት ዋና አላማዉ በሰሜን ምዕራብ ኢትዮጵያ የሚገኙ የቲቢ አምጭ ተህዋስያን፤ማይኮባክቴሪያ የዝርያና መድሃኒትን የመላመድ ሁኔq ማጥናት ነው፡፡

<u>የጥናቱ ጠቀሜq</u>

በሰሜን ምዕራብ ኢትዮጵያ ያለዉን የቲቢ በሽታ አምጪ ባክቴሪያ የመተላለፍ ፤መድሃኒት የመላመድ ሁኔታና የባክቴሪያዉን ዝርያ ማወቅ የተለያዩ የመከላከልና መቆጣጠር እርምጃዎችን ለመዉሰድ እንዲሁም ፈጣን የምርመራና የህክምና ዘዴዎችን ለማስገባት ይረዳል፡፡ከዚህም ባለፈ የበሽታዉን ተጠቂዎች በአግባቡ ለመንከባከብ ይጠቅማል፡፡

<u>የጥናቱ ሂደት</u>

ለዚህ ጥናት እዉን መሆን የእርሶን ተሳትፎ እንፈልጋለን፡፡ በዚህ ጥናት ለመሳተፍ ፈቃደኛ ከሆኑ የስምምነት ቅጹን መረዳትና መፈረም ይጠበቅብዎታል፡፡

በላቦራቶሪ የአክታ ምርመራ የተረጋገጠ የአክታ ናሙና ይሰበሰባል፡፡ የተሰበሰበዉ የአክታ ናሙና አዲስ አበባ ወደሚገኘዉ አርማዉር ሃንሰን ምርምር ተቋም ይሄዳል፡፡ በምርምር ተቋሙ በሚገኘዉ ላቦራቶሪ ጥናቱ ይካሄዳል፡፡ በዚሁ ጥናት የተሳታፊዎችን ስለቲቢ በሽታ ያላቸዉን ግንዛቤ ለማወቅ መጠይቅ ይደረጋል፡፡ የኤች.አይ.ቪ ምርመራ ዉጤት ከህክምና ካርድ ላይ ይረጋገጣል፡፡

<u>ጉዳት/አለመመቸት፡-</u> በዚህ ጥናት የሚደረጉ ሂደቶች ሙሉ በሙሉ በጤና ባለሙያ የሚካሄዱና ከተለመደዉ የአገልግሎት አሰጣጥ የተለዩ ባለመሆናቸዉ ምንም አይነት ምቾት መንሳት ወይም ጉዳት

ባይኖረቸውም ለቃለ ምልልሱ የሚያባክኑት ጊዜ የማህበራዊ ኑሮ ወይም የስራ ሰአትዎን ልጎደዉ ይችላል፡፡፡

<u>የተሳታፊዉ ጥቅሞች፡-</u>

በዚህ ጥናት ምርመራ መድሃኒት የተላመደ የቲቢ አምጭ ባክቴሪያ ከተገኘ በጤና ተቋሙ መዘገብ ላይ በሚገኘዉ አድራሻ መሰረት ከሚመለከተዉ አካል ጋር በመነጋገር ተገቢዉን የህክምናና ክትትል እንዲያገኙ ይደረግልዎታል፡፡ ከዚህም በተጨማሪ የእርሶ ተሳትፎ በህዝባችን ዉስጥ ያለዉን የቲቢ በሽታ ስርጭትና መድሃኒት የተላመዱ የቲቢ በሽታ አምጪ ባክቴሪያ ሁኔታን በማወቅ ወደፊት በሚታቀደዉ የመከለከል፣ የመቆጣጠርና የፈጣን ምርመራና ህክምና ዘዴን ለመቀየስ ከፍተኛ አስተዋጽኦ ይኖረዋል፡፡

<u>ክፍያ</u>፡-በዚህ ጥናት በመሳተፍዎ የሚያገኙት ምንም አይነት ልዩ ክፍያ የለም፡፡ ነገር ግን በቃለ ምልልስ ላባክኑት ጊዜ ማካከሻ ይደረግልዎታል፡፡

<u>ሚስጥራዊነት</u>፡- ማንኛዉም ተሳታፊዉን ገላጭ መረጃ በሚስጥር ይያዛል፡፡በመረጃዉ መስጫ ላይ የቁጥርና የፊደል ስያሜ/ ኮድ/ እንጂ ስም አይቀመጥም፡፡ ሁሉም ሚስጥራዊ ፋይሎች በኮምፕዉተር በይለፍ ቃል ተቆልፈዉ ይያዛሉ፡፡

<u>የተሳታፊዉ መብት፡-</u>

ፈቃደኛ ካልሆኑ በዚህ ጥናት ያለመሳተፍ መብትዎ የተጠበቀ ነዉ፡፡ በዚህም ምክንያት ከሆስፒታሉ ወይም ከጤና ጣቢያዉ በሚያገኙት አገልግሎት ላይ ምንም አይነት ተጽዕኖ አይደርስብዎትም፡፡ ከጥናቱ በማንኛዉም ሰዓት ራስዎን የማግለል መብትዎ የተጠበቀ ነዉ፡፡

<u>ለበለጠ መረጃ፡-</u>

ይህን ጥናት አስመልክቶ ምንም አይነት ጥያቄ ወይም ማብራሪያ ቢያስፈልግዎት፡-

የጥናቱ ተመራማሪ፡ ተክሌ ኤርጊቾ ሎቤ (በአአ ዩኒቨርሲቲ የማስተርስ ተማሪ)

ስልክ፡ 091-175-69-79 ኢሜል፡ teklemicro@gmail.com

ተቆጣጠሪ፡ ደምሰዉ በየነ (አህራ፡ ፒ. ኤች. ዲ.)

ስልክ፡ 091-140-20-60 ኢሜል፡ beyene88@gmail.com

የአርማወር ሃንሰን የምርምር ተቋም የምርምር ስነምግባር ኮሚቴ፤

ስልክ ቁጥር፡0113-481289 አ.አ

ይህ ጥናት የሚካሄደዉ ከአ.አ ዩኒቨርሲቲ ህክምና ፋኩሊቲ፤ማይክሮባዮሎጂ፣ኢሚዉኖሎጂና ፓራሲቶሎጂ ትም/ክፍል እና ከአህራ/አለርት የምርምር ስነምግባር ኮሚቴ ፈቃድ ሲያገኝ ብቻ ነዉ፡፡

ANNEX III

CONSENT FORMAT

I read the information sheet (or it has been read to me). I have understood that this study is about **"Epidemiology and drug resistance pattern of Tuberculosis in North West Ethiopia: Resource limited region"**. I have asked some questions and clarification has been given to me. For this study I have been requested to give three consecutive sputum samples and have an interview. I have been informed that there is no direct benefit to me, but there will be compensation for time spent during interview. The investigator also informed me that all the laboratory results and information collected during the interview will be kept confidential. Moreover, I have also been well informed of my right to withdraw or refuse from participating in this project and this will have no impact on the overall health services. I have been given enough time to think over before I signed this informed consent. It is therefore, with full understanding that I gave my informed consent voluntarily to give sputum sample and to have access of my sero-status from medical records.

Participant's Name : _____signature :_____Date :_____

Investigator's signature : _____ Date :_____

Witness signature 1. _____Date:_____

2. _____ Date: _____

ANNEX IV

<u>የስምምነት ቅፅ</u>

የተሳqፊ ቁጥር፡ _____

የተሳqፊ ስም፡ _____

ስለሚካሄደው ጥናት በቂ መረጃ አግኝቼ የአክq ናሙና ለመስጠት ተስማምቻለሁ፡፡ ከ^ኔ የሚሰበሰበው መረጃ በሙሉ ሚስጥራዊነቱ ^ንደሚጠበቅና በፈለኩት ጊዜና ሰዓት ከጥናቱ ለመውጣት ^ንደምችል ተረድቻለሁ፡፡ ይህም በህክምናየ ሆና ከተቋሙ በማገኘዉ አገልግሎት ላይ ምንም ጉዳት ^ንደማያስከትል ተረድቻለሁ፡፡ በጥናቱ በመሳተፌ ቀጥተኛ የሆነ ጥቅመ ጥቅም ባይኖረዉም በቃለ መጠይቅ ለባከነ ጊዜ ማከሻ ^ንደሚደረግ ተገልያለኛል፡፡ የጥናቱን ሂደት የሚያብራራውን መረጃ አንብቤያለሁ/በሚገባኝ ቋንቋ ተነቦልኛል፡፡ ጥያቄ ለመጠየቅም ^ድሉን አግኝቼ በበቂ ሁኔq መልስ አግኝቻለሁ፡፡ የጥናቱ ተሳqፊ ለመሆንና የኤች.አይ.ቪ. ውጤቱ ከህክምና ካርዴ ላይ ^ንዲqይ ተስማምቻለሁ፡፡

የተሳqፊዉ ፊርማ፡ _____ ቀን:_____

ጥናቱን የሚያካሂደው ፊርማ፡ _____ቀን:_____

የ^ማኝ ፊርማ:_____ቀን:_____

ANNEX V

QUESTIONNAIRES

PART ONE: Patient Identification and Demographic data

Date_____

1.1. Code No: _____ 1.2. Card No. _____

1.3. Hospital/ health centers' ID._____ __

1.4. Address: woreda:_____ Keble: _____ Tel :_____

1.5. Age:_____ 1. 6. Sex: 1.M 2. F

1.7. Occupation

1. Farmer 2. Merchant 3.Government employee 4. Other_____ (specify)

1.8. Area of residence:

1. Urban 2. Rural

1.9. Religion

1. Muslim 2. Christian 2.1. Orthodox 2.2. Protestant 2.3. Catholic

3. Other _____(specify)

1.10. Educational level

1. Illiterate 2. Elementary 3. High school 4. Diploma 5. University degree

5. Others_____(specify)

1.11. Income level (ETB/ yr)

1. <200 2. 500 3. 500-1500 4. 1500-3000 5. 3000-5000 6. > 5000

1.12. How far is health center or hospital from your residence? (Describe distance on foot travel)

1. Half a day on foot 2. One day on foot 3. One and half day on foot 4. Two days on foot 5. Other_____ (specify)

1.13. Number of family: _____

1.14. Weight: _____ (in kg)

1.15. Height: ____ (meter/centimeters)

1.16. Ethnic group

1. Amhara 2. Oromo 3. Tigray

4. Shinasha 5. Gumuz 6. Other _____ (specify)

PART TWO: Clinical data

2.1. Symptom of TB

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

2.2. Previous exposure of anti-tuberculosis drugs: A. yes B. No

If yes, 1. Complete 2. Default 3. Re-treatment 4. Relapse

2.3. Mention drug regimen: (to be filled by interviewer only)

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

2.4. Did you ever missed taking anti- TB drug properly? 1. Yes 2. No

If yes, how long? _____(Specify)

PART THREE: Contact History

3.1. Do you have any contact history with suspected TB Patients? 1. Yes 2. No if yes,

1. Family 2. Friend 3. Neighbor 4.Other _____(specify)

3.2. Is there anyone in your family that had been infected with *M. tuberculosis*? 1. Yes 2. No if yes,

1. Wife 2. Husbands 3.Children
4. Father 5. Mother 6. Other_____ (specify)

PART FOUR: TB Knowledge and Awareness

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

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

4.2. What are the signs and symptoms of TB?

1. Cough<2wks 2. Cough that lasts longer than 2 Wks 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)

4.3. How can a person get TB?

1. By air droplets when a person with TB coughs or sneezes 2. Through handshakes

3. By sharing dishes 4. Drinking unpasteurized milk 5. Curse of God

6. Other _____(specify)

4.4. 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. Balanced diet 7. Do not know 8. Other _____ (specify).

4.5. 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 moderately expensive

4.6. Are you taking TB medicines? 1. Yes 2. No 3. Never started

4.7. How frequent are you supposed to take your medicines?

1. Daily 2. Once weekly 3. Twice weekly 4. Three times weekly

5. Other: _____(specify)

4.8. When do you expected to stop taking anti tuberculosis drugs? After (_____) Months

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

1. Anybody 2. Only poor peoples 3. Only homeless peoples

4. Only alcoholics 5. Only drug users 6. Only HIV/AIDS patients

7. Only people in prison 8. Other _____(specify)

4.10. Can TB be cured? 1. Yes 2. No

4.11. How can someone with TB be cured?

1. Herbal remedies 2. Rest without medicine 3. Praying

4. Anti-tuberculosis drugs given by health institute 5. Do not know

6. Other :_____(specify)

PART FIVE: TB Attitudes

5.1. Do you think you can get TB?

1. Yes, why? _____

2. No, why? _____

5.2. In your opinion, how serious disease is TB?

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

5.3. How serious problem you think TB in your region?

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

5.4. 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)

5.5. Who would you talk 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)

5.6. 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)

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

PART SIX. Health-Seeking Behavior

6.1. Where do you usually go if you are sick?

1. Private clinic 2. Government clinic or hospital 3. Traditional healer

4. Church/ mosque 5. Other: _____(specify)

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

1. Twice a year or more 2. Once per year 3. At least twice in past 5yrs

4. Once in past 5 years 5. Never in past 5 years 6. Other: _____(specify)

6.3. 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

4. Do not trust medical workers 5. Others: _____(specify)

PART SEVEN: TB Awareness and Sources of Information

7.1. Do you feel well informed about TB? 1. Yes 2. No

7.2. Do you wish you could get more information about TB? 1. Yes 2. No

7.3. 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. Religious leaders 8. Teachers 9. Other: _____ (specify)

Name of the interviewer:_____Signature:_____Date:_____

THANK YOU VERY MUCH FOR YOUR PARTICIPATION IN OUR STUDY!!

ANNEX VI

የተሳታፊዎች መጠየቂያ ቅጽ ቀን፡.....

ክፍል 1፡ የተሳታፊዉ መለያና ማህበረሰባዊ ሁኔታ መጠይቆች

1.1፡ **የኮድ ቁጥር፡**..... 1.2፡ **የካርድ ቁጥር፡**.....

1.3፡ **የጤና ተቋሙ መለያ ቁጥር፡**.....

1.4፡ **አድራሻ፡ ዞን፡**..... **ወረዳ፡**..... **ቀበሌ፡**.....

1.5፡ **ፆታ፡** 1.**ወንድ** 2.**ሴት**

1.6፡ **እድሜ፡**.....

1.7፡ **የስራ ሁኔታ፡**

1.**አርሶ አደር** 2. **ነጋዴ** 3. **የመንግስት ሰራተኛ** 4. **ሌላ ካለ ይገለፅ፡**.....

1.8፡ **የመኖሪያ አከባቢ፡** 1. **ከተማ** 2. **ገጠር**

1.9፡ **ሃይማኖት፡** 1.**ሙስሊም** 2.**ክርስቲያን** 2.1.**አርቶዶክስ** 2.2.**ፕሮቴስታንት** 2.3.**ካቶሊክ** 3. **ሌላ ካለ ይገለፅ፡**.....

1.10፡ **የትምህርት ደረጃ፡**

1. **አልተማርኩም** 2. 1-4 **ክፍል** 3. 5-8 **ክፍል** 4. 9-12 **ክፍል**

5. **ዲፕሎማ** 6. **ዲግሪና ከዚያ በላይ**

1.11፡ **የገቢ መጠን /በኢት. ብር በአመት/**

1. **ከእጅ ወደ አፍ /ከ**200.00 **ብር በታች/** 2. 500.00 3. 501.00-1500.00

4. 1501.00-3000.00 5. 3001.00-5000.00 6. **ከ**5000.00 **በላይ**

1.12፡ **በአቅራቢያዎ የሚገኘዉ የጤና ተቋም ከመኖሪያ አድራሻዎ በእግር ጉዞ ምን ያህል ያስኬዳል?**

1.**ግማሽ ቀን** 2.**አንድ ቀን** 3.**አንድ ቀን ከግማሽ** 4.**ሁለት ቀን** 5. **ሌላ ካለ ይገለፅ፡**.....

1.13፡ **የቤተሰብ አባላት ብዛት፡**.....

1.14፡ **ክብደት፡**.....**በኪ.ግራም** 1.15፡ **ቁመት፡** .....**በሜትር**

1.16፡ **ብሔር፡**1.**አማራ** 2.**ኦሮሞ** 3.**ትግራይ** 4.**ሸናሻ** 5.**ጉሙዝ**6. **ሌላ ካለ ይገለፅ፡**.....

17፡ **የጋብቻ ሁኔታ፡** 1.**ያገባ/ች** 2. **ያላገባ/ች** 3. **የተፋታ/ች** 4. **ሌላ ካለ ይገለጽ፡**.....

ክፍል 2፡ የህክምና መረጃ

2.1፡ **የቲቢ በሽታ ምልክቶች**

1. **ከሁለት ሳምንት በላይ የቆየ ሳል** 2. **የአክታ መዉጣት**

3. **ሌሊት ማላብ** 4. **ድካም** 5. **የትንፋሽ ማጠር**

6. **የክብደት መቀነስ** 7. **ትኩሳት** 8.**አላዉቅም** 9. **ሌላ ካለ ይገለፅ፡**.....

2.2፡ **ከዚህ በፊት የፀረ-ቲቢ መድሃኒት ወስደዉ ያዉቃሉ?** 1.**አዎ** 2.**አይደለም፣ አዎ ከሆነ መልስዎ**

1. **መድሃኒቱን ጨርሻለሁ** 2. **መድሃኒቱን አቋርጫለሁ** 3. **የድጋሚ ህክምና** 4. **አገርሽቶብኛል**

2.3፡ **የወሰዱት የመድሃኒት አይነት ይጠቀስ፡፡/ በጠያቂዉ የሚሞላ/**

1. **አዲስ፡**..... 2. **የድጋሚ ህክምና፡**..... 3. **ተመላላሽ፡**.....
4. **ያቋረጡ፡**..... 5. **ያገረሸባቸዉ፡**.....

2.4፡ **የፀረ-ቲቢን መድሃኒት በትክክል ያልወሰዱበትን ጊዜ ያስታዉሳሉ?**1. **አዎ** 2. **አይደለም**

መልስዎ አዎ ከሆነ፣ ለምን ያህል ጊዜ?ይገለፅ.....

ክፍል 3፡ የንክኪ ሁኔታ፡

3.1፡ **ከዚህ በፊት ከቲቢ ህመምተኛ ጋር ንክኪ ኖሮዎት ያዉቃል?** 1. **አዎ** 2. **አይደለም፣ መልስዎ አዎ ከሆነ** 1. **ቤተሰብ** 2. **ጓደኛ** 3. **ጎረቤት** 4. **ሌላ ካለ ይገለፅ፡**.....

3.2፡ **በቤትዎ ዉስጥ በቲቢ በሽታ የተያዘ ሰዉ አለ?**1. **አዎ** 2. **አይደለም፣ አዎ ከሆነ መልስዎ**

1. **ባል** 2. **ሚስት** 3.**ልጅ** 4. **አባት** 5. **እናት** 6. **ሌላ ካለ ይገለፅ፡**.....

ክፍል 4፡ ስለቲቢ በሽታ እዉቀትና ግንዛቤ

4.1፡ **በመጀመሪያ ስለቲቢ በሽታ የሰሙት ከየት ነዉ?ከአንድ በላይ መልስ መስጠት ይችላሉ፡፡**

1. **ከጋዜጣ** 2.**ከሬድዮ** 3. **ከቴሌቪዥን** 4.**ከተለጣፊ ወረቀት** 5. **ከበራሪ ወረቀት**
6. **ከጤና ባለሙያ** 7. **ከቤተሰብ** 8.**ከሃይማኖት መሪዎች** 9. **ከመምህራን**
10. **ሌላ ካለ ይገለፅ፡**.....

4.2፡ **የቲቢ በሽታ ምልክቶች ምን ምን ናቸዉ?**

1. **ከሁለት ሳምንት ያነሰ ሳል** 2. **ከሁለት ሳምንት ያለፈ ሳል** 3.**ደረት ላይ የህመም ስሜት** 4. **የትንፋሽ ማጠር** 5. **ድካም ድካም ማለት** 6.**የክብደት መቀነስ**
7. **ማቅለሽለሽ** 8. **ከባድ ራስ ምታት** 9. **ትኩሳት** 10. **አላዉቅም** 11. **ሌላ ካለ ይገለፅ፡**.....

4.3፡ **የቲቢ በሽታ የሚመጣዉ ከምንድን ነዉ ብለዉ ያስባሉ?**

1. **ከባክቴሪያ** 2. **ከቫይረስ** 3. **ከፈንገስ** 4. **አይታወቅም**

5. **በእርግማን** 5. **ሌላ ካለ ይገለፅ፡**.....

4.4፡ የቲቢ በሽታ እንዴት ሊተላለፍ ይችላል ብለዉ ያስባሉ?

1. የቲቢ በሽታ የያዘዉ ሰዉ በሚያስልበትና በሚያስነጥስበት ጊዜ በአየር አማካይነት

2. እጅ በመጨባበጥ 3. አብሮ በመመገብ 4. ያልፈላ ወተት በመጠጣት 5. በእርግማን

6. ሌላ ካለ ይገለፅ፡.....

4.5፡ የቲቢ በሽታን እንዴት መከላከል ይቻላል?

1. በሚያስልበት ወይም በሚያስነጥስበት ጊዜ አፍና አፍንጫን በመሐረብ /በጨርቅ/ በመሸፈን

2. እጅ ባለመጨባበጥ 3. አብሮ ባለመብላት 4. መስኮቶችን በመዝጋት 5. በፀሎት

6. የተመጣጠነ ምግብ በመመገብ 7. አላዉቅም 8. ሌላ ካለ ይገለፅ፡.....

4.6፡ በአገራችን የቲቢ በሽታ ምርመራና ህክምና ምን ያህል ዉድ ነዉ ብለዉ ያስባሉ?

1.ነፃ ነዉ 2.ዉድ አይደለም 3. በመጠኑም ቢሆን ዉድ ነዉ 4. በጣም ዉድ ነዉ 5.አላዉቅም

4.7፡ በአሁኑ ጊዜ የፀረ-ቲቢ መድሃኒት እየወሰዱ ነዉ? 1.አዎ 2. አይደለም 3.ወስጀ አላዉቅም

4.8፡ የፀረ-ቲቢ መድሃኒት መወሰድ ያለበት በምን ያህል የጊዜ ልዩነት ነዉ?

1. በየቀኑ 2.በሳምንት አንዴ 3.በሳምንት ሁለቴ 4.በሳምንት ሶስቴ 5. ሌላ ካለ ይገለፅ፡.....

4.9፡ የፀረ-ቲቢ መድሃኒት መዉሰድ ማቆም ያለብዎት መቼ ነዉ?ከ.....ወር በኃላ

4.10፡ በእርስዎ አመለካከት በቲቢ በሽታ ማን ሊያዝ ይችላል ብለዉ ያስባሉ?

1. ማንኛዉም ሰዉ ሊያዝ ይችላል። 2. ድሃ ብቻ 3.ቤት የሌላቸዉ 4. ስካራሞች

5. የተለያዩ መድሃኒቶችን የሚጠቀሙ 6.ኤች.አይ.ቪ/ኤድስ በሽተኞች ብቻ

7. እስረኞች 8. ሌላ ካለ ይገለፅ፡.....

4.11፡ የቲቢ በሽታ ይድናል? 1. አዎ 2. አይደለም

4.12፡ የቲቢ በሽታ የያዘዉ ሰዉ እንዴት ሊድን ይችላል?

1. መድሃኒትነት ያላቸዉን ተክሎች በመጠቀም 2. ያለመድሃኒት በቂ እረፍት በማድረግ

3. በመፀለይ 4. ከህክምና ተቋመት የፀረ-ቲቢ መድሃኒት በመዉሰድ 5. አላዉቅም

6. ሌላ ካለ ይገለፅ፡.....

ክፍል 5፡ስለቲቢ በሽታ አመለካከት

5.1፡ **የቲቢ በሽታ ሊይዘኝ ይችላል ብለዉ ያስባሉ?**

1. **አዎ፣ ለምን?**..... 2. **አይደለም፣ ለምን?**.....

5.2፡ **በእርሶ አመለካከት የቲቢ በሽታ ምን ያህል አሳሳቢ ነዉ?**

1. **በጣም አሳሳቢ ነዉ** 2. **በመጠኑ አሳሳቢ ነዉ** 3. **ምንም አሳሳቢ አይደለም**

5.3፡ **በክልልዎ የቲቢ በሽታ ስርጭት ምን ያህል አሳሳቢ ነዉ?**

1. **በጣም አሳሳቢ ነዉ** 2. **በመጠኑ አሳሳቢ ነዉ** 3. **ምንም አሳሳቢ አይደለም**

5.4፡ **እርስዎ የቲቢ በሽተኛ ቢሆኑ/በመሆንዎ/ ምን አይነት ስሜት ይሰማዎታል?**

1. **ፍርሃት** 2. **ግርምት** 3. **ዉርደት** 4. **ሃዘን** 5.**ሌላ ካለ ይገለፅ፡**.....

5.5፡ **የቲቢ በሽተኛ መሆንዎን ሲያዉቁ ከማን ጋር ማዉራት ይፈልጋሉ?**

1.**ከጤና በለሙያ ጋር** 2.**ከትዳር ጓደኛ ጋር** 3. **ለወላጆች** 4. **ለቅርብ ጓደኛ**

5. **ለማንም አልናገርም** 6. **ሌላ ካለ ይገለፅ፡**.....

5.6፡ **የቲቢ በሽታ ምልክቶች ያለብዎት ቢመስልዎት ምን ያደርጋሉ?**

1. **ወደ ጤና ተቋም እሄዳለሁ** 2. **ወደ ፋርማሲ/መድሃኒት ቤቶች/ እሄዳለሁ**

3. **ወደ ባህላዊ ህክምና እሄዳለሁ** 4. **ራሴን ለማከም እሞክራለሁ** 5. **ሌላ ካለ ይገለፅ፡**.....

5.7፡**ስለቲቢ በሽታ ሲያስቡ በጣም የሚያስጨንቅዎት ምንድን ነዉ?**.....

ክፍል 6፡ የጤና አገልግሎት አጠቃቀም ሁኔታ

6.1፡ **በሚታመሙበት ጊዜ ምን ያደርጋሉ?**

1. **ወደ ግል ክሊኒክ እሄዳለሁ** 2. **ወደ መንግስት ጤና ተቋም እሄዳለሁ**

3. **የባህል ጤና አገልግሎት ወደሚሰጥበት እሄዳለሁ** 4. **ወደ እምነት ተቋማት እሄዳለሁ**

5. **ሌላ ካለ ይገለፅ፡**.....

6.2፡ **ለጤና ምርመራ ወደ ጤና ተቋማት በአመት ስንት ጊዜ ይሄዳሉ?**

1. **ሁለቴና ከዚያ በላይ** 2.**በአመት አንዴ** 3.**ባለፉት አምስት አመታት ሁለቴ ብቻ**

4. **ባለፉት አምስት አመታት አንዴ ብቻ** 5. **ባለፉት አምስት አመታት ሄጄ አላዉቅም**

6. **ሌላ ካለ ይገለፅ፡**.....

6.3፡ ወደ ጤና ተቋማት የማይሄዱባቸዉ ምክንያቶች ምንምን ናቸዉ?

1. የት መሄድ እንዳለብኝ ስለማላዉቅ 2. ዋጋዉን ስለማልችል 3. የመጓጓዣ ችግር/የቦታ ርቃት/

4. በጤና ባለሙያዉ ላይ እምነት ማጣት 5. ሌላ ካለ ይገለፅ፡.....

<u>ክፍል 7፡ ስለቲቢ በሽታ ግንዛቤና የመረጃ ምንጭ</u>

7.1፡ ስለቲቢ በሽታ ስነገር ደስ ይልዎታል? 1. አዎ 2. አይደለም

7.2፡ ስለቲቢ በሽታ ብዙ ማወቅ ይፈልጋሉ?1. አዎ 2. አይደለም

7.3፡ ለእርሶና እንደእርሶ ላሉት ሌሎች ሰዎች የትኛዉ የመረጃ ምንጭ ስለቲቢ በሽታ መረጃ ለማስተላለፍ ትክክለኛና ቀጥተኛ ነዉ ብለዉ ያስባሉ?

1.ጋዜጣ 2. ሬዲዮ 3.ቴሌቭዥን 4.የሚለጠፉ ጹሁፎች 5. በራሪ ወረቀቶች

6.የጤና ባለሙያዎች 7.የሃይማኖት መሪዎች 8.መምህራን 9.ሌላ ካለ ይገለፅ፡.....

ዉድ ጊዜዎን ሰዉተዉ በጥናታችን ስለተሳተፉ ከልብ እናመሰግናለን፨

የጠያቂዉ ስም፡..... **ፊርማ፡**.....

ቀን፡.....

ANNEX VII

LABORATORY INFORMATION SHEET

Name of Health Institution: _____ Date: _____

Patient's register number: _____

Source of specimen: **Pulmonary**

Reason for examination: **Diagnosis**

Specimen identification number: _____ Date: _____

LABORATORY RESULTS: FINAL REPORT

Laboratory serial number: _____ Date specimen received: _____

1. Microscopy results

Staining method: **Ziehl-Neelsen** ☐

Negative	☐	1 +	☐
1-9 AFB	☐	2+	☐
Not done	☐	3+	☐

2. Culture results

Culture method: _____

No growth	☐	1+	☐
Contaminated	☐	2+	☐
Not done	☐	3+	☐
1-19 colonies	☐	4+	☐

3. Culture identification

Growth rate: _____ Colony morphology:_____

Niacin production: Positive ☐ Negative ☐

Nitrate production: Positive ☐ Negative ☐

Catalase test: Positive ☐ Negative ☐

Culture identified as: *Mycobacterium tuberculosis*: ☐ MOTT: ☐

Name: _____**Signature**: _____ **Date**: _____

4. Drug susceptibility result on LJ medium

Specimen	Date o f specimen collection	Patient ID No	Lab. serial number	STM	INH	RIF	EMB	Remark
1.								
2.								
3.								
”								
”								
143.								

NB: Put “**R**” *If it is Resistant*: “S”: *If it is Susceptible*: “**C**”: *If it is Contaminated* “**ND**”: *If it is* not done

Name: _____**Signature**: _____ **Date**: _____

ANNEX VIII

DECLARATION SHEET

We the undersigned agreed to accept responsibility for the scientific ethics and technical conduct of the biomedical research and certify that the MSc thesis is prepared and submitted by Tekle Airgecho Lobie on " **Epidemiology and Drug resistance pattern of *Mycobacterium tuberculosis* in Northwest Ethiopia; Resource limited setting**" in partial fulfillment of the requirements for Degree of Master of Science in Medical Microbiology. The thesis complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Principal investigator:

Tekle Airgecho Lobie (BSc, MSc Candidate; AAU)

Signature: _____ Date: _____

Advisors/co-investigators

1. **Yimtubezinash Woldeamanuel** (MD, MSc, PhD, Associate professor)

Signature: _____ Date: _____

2. **Daniel Asrat** (MD, MSc, PhD, Associate professor)

Signature: _____ Date: _____