Tuberculosis in Farmers and Their Cattle in Smallholder Farming System in South Gondar Zone of Northwest Ethiopia: Epidemiology and Drug Sensitivity Profiles

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ABSTRACT

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Tuberculosis (TB), an infectious disease caused by *Mycobacterium tuberculosis* complex (MTBC), remains a public health problem in Ethiopia. However, there is still shortage of epidemiological data in different parts of the country, particularly, in the rural and semi-urban communities. Hence, the present study was conducted to investigate the epidemiology of TB in farmers and their cattle in South Gondar Zone, northwest Ethiopia. In addition, the drug sensitivity profile of *M. tuberculosis* isolated from TB cases was evaluated. The human aspect of the study was conducted on 2953 individuals using clinical and bacteriological examinations.Region of difference (RD) 9-based polymerase chain reaction (PCR) and spoligotyping were done on the MTBC isolates. Drug sensitivity pattern of the isolates was analyzed using the GenoType MTBDR*plus* assay. The animal aspect of the study was conducted on 95 herds consisting of 476 cattle using the single intra-dermal cervical comparative tuberculin test (SICCTT). The study determined TB prevalence of 6.3% (186/2953) in humans visiting health facilities in the study area. Some of the patientsô demographic characteristics (patientsô origin, χ^2 value: 62.80; p<0.001 and age group,χ^2 value: 35.46; p<0.001) were found to be significantly associated risk factors for TB infection. Out of 96 *M. tuberculosis* isolates spoligotyped, 35 spoligotype patterns were identified, of which 22 were shared typesand consisted of 79 isolates. Thirteen of these patterns were clustered consisting of 74 isolates making the clustering rate of 77.1%. The dominant shared international types (SITs) were SIT53 (Lineage 4), SIT149 (Lineage 4) and SIT428 (Lineage 3), each consisting of 18.8%, 12.5% and 12.5% of the total isolates, respectively. The SIT 428 was found to be specific to the study area and associated with EPTB. From the culture positive *M. tuberculosis* isolates, 18.0% (20 /111) were resistant to at least one of the two most effective first-line anti-TB drugs, isoniazid (INH) and rifampicin (RIF). Multi-drug resistant (MDR) TB was detected in 1.8% (2/111) of the cases. The high proportion of drug resistant *M. tuberculosis* strains in the study area indicates the need for an increased effort to strengthen TB control program in the study area.The animal and herd prevalence of bovine TB were 1.5% (7/476) and 7.4% (7/95), respectively with the odds of bovine TB in cattle owned by TB positive households being slightly higher than those owned by TB free households. This may suggest the zoonotic transmission potential of TB from domestic cattle. Overall, the study revealed a relatively high prevalence of TB in humans and all human isolates were *M. tuberculosis* and no *M. bovis* was isolated from the study population. The failure to detect *M. bovis* in the study could be due to the low prevalence of bovine TB in the area.

Key words: Tuberculosis, Epidemiology, Drug sensitivity, Zoonoses,South Gondar, Ethiopia.

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List of Abbreviations

AC	Amplification control
AFB	Acid-fast bacilli
AFRI;	Africa
AG	Arabinogalacton
AIDS	Acquired immune deficiency syndrom
ALIPB	Aklilu Lemma institute of pathobiology
BCG	Bacilli Calmette-Guerin
BTB	Bovine tuberculosis
CAS	Central Asian
CBN	Conformal Bayesian network
CC	Conjugate control
CSA	Central statistics agency
DNA	Deoxy-ribonucleic acid
DOTS	Directly observed treatment short course
DPA	D-arabinofuranosyl-1-monophosphoryl decaprenol
DR	Direct repeat
DR-TB	Drug resistant-TB
DST	Drug sensitivity test
EA	Euro-American
EAI	East African-Indian
EAS	East Asian
EMB	Ethambutol
EPTB	Extrapulmonary tuberculosis
FAO	Food and agricultural organization
FDRE	Federal democratic republic of Ethiopia
FMoH	Federal ministry of health

FNA	Fine needle aspirate
GC	Guanine-cytosin
H:	Haarlem
HBCs	High burden countries
HIV	Human immunodeficiency virus
INH	Isoniazid
IO	Indo-Oceanic
IS	Insertion sequence
KBBN	Knowledge based Bayesian network.
LAM	Latin American-Mediterrane
LSPs	Large sequence polymorphisms
MDR	Multidrug resistance
MIRUs	Mycobacterial interspersed repetitive units
MPTR	Major polymorphic tandem repeats
MTBC	*Mycobacterium tuberculosis* complex
MUT	Mutant.
NALC	N-acetyl-L-cysteine
NTM	Non-tuberculin mycobacteria
OIE	Organisation for animal health
OR	Orphan
PBS	Phosphate buffer saline
PCR	Polymeraze chain reaction
POA	Pyrazinoic acid
PPD	Purified protein derivative
PTB	Pulmonary tuberculosis
PZA	Pyrazinamide
PZase	Pyrazinamidase/nicotinamidase

RFLP	Restriction fragment length polymorphism
RMP	Rifampin
RRDR	Rifampin resistance determining region
rRNA	Ribosomal ribonucleic acid
RR-TB	Rifampin resistant-tuberculosis
SDGs	Sustainable development goals
SICCTT	Single intra-dermal cervical comparative tuberculin test
SIT	Sharedinternational type
SPolDB4	Spoligotype data base 4
STR	Streptomycin
TB	Tuberculosis
tRNAs	Transfer ribosomal nucleic acids
UN	United Nations
VNTR	Variable-number tandem repeat
WHO	World health organization
WT	Wild type
XDR	Extensively drug-resistant
ZN	Ziehl-Neelsen

1. INTRODUCTION

1.1. General

Tuberculosis (TB) is an infectious disease that has affected humans for thousands of years.*M. tuberculosis*, the bacterium that causes TB is an obligate human pathogen. It is transmitted by aerosols containing infectious *M. tuberculosis* released from the lungs of infected individuals upon coughing. TB predominantly affects the lungs, but can occur in any tissue (Young *et al.* 2009).

Recently, TB became the main cause of death due to an infectious agent, ranked above HIV/AIDS (WHO, 2018). In 2017, an estimated 10.0 million incident cases of TB were reported globally, which is equivalent to 133 cases per 100, 000 population (WHO, 2018). By the same year, there were an estimated 1.3 million deaths among HIV-negative people due to the disease, and an additional 300 000 deaths from TB among HIV-positive people (WHO, 2018).

The emergence of HIV/AIDS coupled with socio-demographic factors have made a significant impact on TB epidemics, especially in developing countries such as Ethiopia (Wood *et al*., 2010; Amare *et al*., 2013; Addis *et al*., 2015; Mumpe-Mwanja*et al*., 2015; Alemayehu *et al*., 2017).TB control activities require regular measurement of the absolute burden of disease and proper identification of the potential risk factors to monitor trends and improve understanding of its epidemiology (WHO, 2011). However, in many high burden countries (HBCs), TB statistics is often unreliable due to gaps in recording notifications due to poor registration systems (WHO, 2011). A study in Amhara region, northwest Ethiopia also revealed that poor

TB documentation and reporting were major challenges for control program of the disease (Gebreegziabher*et al*.,2016).

Although the major cause of human TB is*M. tuberculosis*, considerable proportion of it occurs due to *M. bovis* (bovine tuberculosis; BTB). BTB is the main zoonotic health concern transmitted to humans largely through consumption of raw milk and other products obtained from infected cattle and/or occasionally through respiratory route (Óreilly and Daborn, 1995). Studies suggested that in countries where pasteurization of milk is rare and BTB is common, 10 to 15% TB cases in humans are caused by *M. bovis* (Ashford *et al*., 2001). In 2016, an estimated 147, 000 new cases of zoonotic TB and 12, 500 deaths due to the disease was reported in humans. Among these, the African region comprised largest number of incidence (49.5%) and deaths (74.4%), due to zoonotic TB (WHO, 2017).

In Ethiopia, the available reports show that the prevalence of BTB ranges from 3.4% in smallholder production systems to 50% in intensive dairy production systems (Ameni *et al*., 2001, 2007; Berg *et al*., 2009). However,previous studies on BTB focused on specific target areas of central highlands and pastoral communities of southern and south-eastern parts of Ethiopia.In the Amhara region of Ethiopia, particularly in South Gondar zone, little is known about the situation with animal TB. There is no adequate data on the detection of BTB either on live animals or in the abattoirs.

Clinically, TB caused by *M. bovis* can only be differentiated from TB caused by *M. tuberculosis* specificallyy by laboratory methods (Cosivi *et al*., 1998). However, the routinely used sputum smear microscopy TB diagnosis, due to its low sensitivity, has contributed for the unknown well defined role of *M. bovis* infection to TB cases in humans in Africa (Ayele *et al*., 2004). Therefore, to make an evidence-based intervention against the disease,more effort is needed in the genaration of baseline data through real time surveillance and the use of diagnostic tools with better resolution than smear microscopy. Awarness creation concerning food safety and cattle husbandary is also an important part of the overall TB control strategy in Ethiopia, particularly in smallholder dairy farming systems in rural and semi urban settings, where human and cattle live in close proximity and raw milk consumption is part of the communityös livelihood.

Understanding the molecular epidemiology of causative agents is a powerful approach in providing correct distinction between relapse and reinfection, identifying the source of infection and provides unique insights into the transmission dynamics of *M. tuberculosis* (Savine *et al*., 2002; Stragier *et al*., 2005). However, in most developing countries, particularly in Ethiopia, direct smear microscopy of sputum was the most common method used for the routine detection of *M. tuberculosis* inhealth facilities. Hence, there is no adequate information on the type of *M.tuberculosis* strains circulating in the community. Drug resistant TB (DR-TB) remained a public health threat with 490,000 million cases of multidrug resistant-TB (MDR-TB) and an additonal 110, 000 cases that were susceptible to isoniazid (INH) but resistant to rifampicin (RIF), RR-TB; the most effective first-line anti-TB drug (WHO, 2017). According to the same report, an estimated 4.1% of new cases and 19% of previously treated cases had MDR/RR-TB at the end of 2016. In this respect, for the same year, Ethiopia has reported lower level of DR-TB cases than the global average (2.7% resistance in newly dignosed and 14% of previously treated cases)(WHO, 2017).However, the above facts suggested that drug resistance remained a public health problem in the effort to control TB in the country.

According to the WHO defenition, MDR-TB is a form of DR-TB in which *M. tuberculosis* can no longer be killed at least by the two most effective first-line anti-TB drugs (RIF and INH). Nowadays, WHO recommended resistance to RIF(RR-TB) can be considered as MDR and should be treated with second-line anti-TB drugs. A study conducted in Addis Ababa, reported 72.9% resistance to at least one of the first-line anti-TB drugs (Abate *et al*., 2012). A similar study in Oromia region reported 33% MDR-TB cases (Mulisa *et al*., 2015). Studies from other parts of Africa such as South-Africa, Uganda,Zambia, Tanzania, Zimbabwe and Kenya, reported significant proportions of *M. tuberculosis* isolates that were resistant to at least one of the first-line anti-TB drugs (Silaigwana *et al*., 2012; Lukoye *et al*., 2013; Sagonda *et al*., 2014; Hoza *et al*., 2015; Kapata *et al*., 2015;Ombura *et al*., 2016).

Several studies reported patient and clinical characteristics such as previous treatment cases, age, sex, occupation, previous history of contact to TB patients and forms of TB as predictors for development of anti-TB drug resistance (Bazira *et al*., 2011; Abate *et al*., 2012; Biadglegne *et al*., 2014; Seyoum *et al*., 2014;Mulisa *et al*., 2015). Considerable number of previous studies elsewhere in the world associated genotypes of *M. tuberculosis* with development of drug resistance (Kibiki *et al*., 2007; Bazira *et al*., 2011; Ayaz *et al*., 2012; Bedewi *et al*., 2016; Brhane *et al*., 2017; Mathuria *et al*., 2017).

However, lack of enough concern to undertake TB research activities and generating base line information on TB situation in humans and livestock in smallholder farms, the majority of them are in rural and semi-urban settings, hampered the delivery of proper intervention against TB in the country. In addition, incidence and prevalence rates of human TB due to *M. .bovis* ismostly unknown. Therefore, to achieve the national target in TB control program the current challanges and top priority gaps should be adressed, especially in endemic and rural settings of the country such as the present study area.

1.2. Smallholder dairy farming systems and the risk of zoonotic diseases

According to a report from the Central Statistics Agency (CSA) of Ethiopia, currently, the country has about 59.5 million cattle, out of which, 55.5% are females and the majority (62.95%) are in the age range of 3 to less than 10 years (CSA, 2017). Based on the same report, about 98.2% of the total cattle population is local breeds, the remaining are hybrid (1.62%)and exotic (0.18%) breeds. From the total cattle population at the end of 2016/17, more than 11.8 million are milking cows (CSA, 2017).

In Ethiopia and other parts of Africa, smallholder dairy farming systems are common and various classifications of them have been stated (Tsehay *et al*., 2002; Azage *et al*., 2013;Gizaw *et al*., 2017). Though various criteria were used to classify smallholder dairy farming, majority of the classifications were based on demographic settings which included rural, peri-urban and urban systems.

The smallholder dairy farms like pastorialists, agro-pastorialists and mixed crop-livestock producers which are very common in rural areas are part of the subsistence farming, usually not market oriented (Gillah *et al*., 2012). In contrast, the periurban and urban smallholder farms are contributing to fill the demand-supply gap of milk and milk product consumption in urban centers (Gillah *et al*., 2012).

Despite the importance of smallholder dairy farms in supporting the livelihood of the producers, there are potential public hralth concerns regarding milk-borne diseases (such as brucellosis, enterotoxaemia and tuberculosis) and hygiene in raw milk and traditional milk products (Shirima *et al*., 2003; Bertu *et al*. 2010).

In Ethiopia and other developing nations, smallholder dairy farmersô awareness on cattle and milk-borne zoonoses as well as hygienic milking practices were found to be low (Getachew, 2003; Duguma and Janssens, 2015). Unhygenic hand milking, poor collection and storage of milk, raw milk consumption habits and lack of a practice of medical examination of milkers and poor veterinary and extension services were reported to be some of the potential public health risk factors in smallholder dairy farming systems in Ethiopia (Zelalem and Faye 2006; Belay *et al*., 2012).

Information about milking hygiene practices and farmersô awareness on cattle and milk-borne zoonoses remain scarce in South Gondar areas. This lack of enough information could result in public health risks and economic losses in the study area. Hence, improving the awarness of farmers in the study area on zoonotic transmission of diseases to humans will improve the quality of raw milk and reduce the risk of human health threats.

1.3. Global public health burden of TB

Globally, TB becomes one of the top 10 causes of death, and the leading cause from a single infectious agent (above HIV/AIDS). In 2017, there were an estimated 1.3 million deaths among HIV-negative people due to the disease, and an additional 300 000 deaths from TB among HIV-positive people (WHO, 2018).

In 2017, an estimated 10.0 million incident cases of TB were reported globally,which is equivalent to 133 cases per 100, 000 population (WHO, 2018). The same report also indicated that the majority of the estimated number of cases occurred in the South-East Asia Region (44%), the African Region (25%) and the Western Pacific Region (18%)(Figure 1).

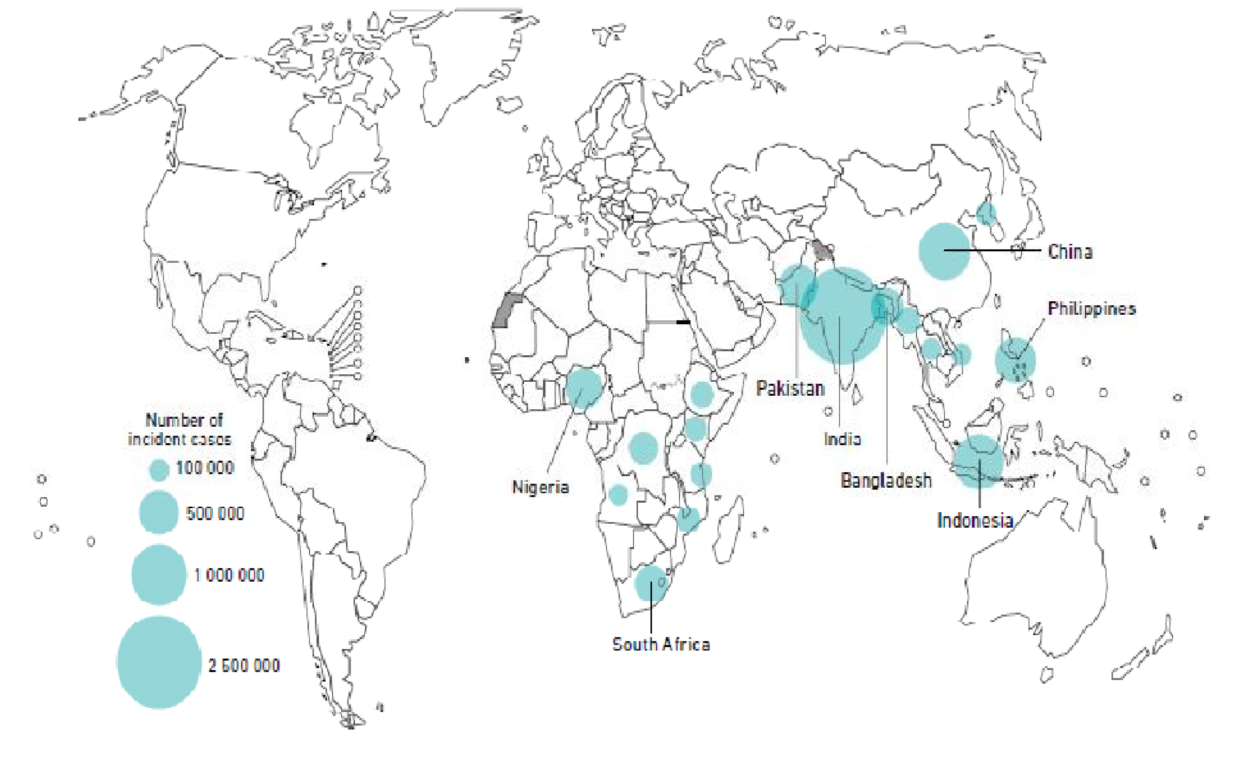

Figure 1.Estimated TB incidence in 2017 for countries with at least 100 000 incident cases(WHO, 2018).Eight of the 30 high TB burden countries accounted for two thirds of the global total estimated incident cases of TB: India (27%), China (9%), Indonesia (8%), the Philippines (6%), Pakistan (5%), Nigeria (4%), Bangladesh (4%) and South Africa (3%).

1.3.1. TB burden in Ethiopia

Despite the long journey to control programms and a slight decline of cases in the last decade, TB is having a major health burden and economic impact in Ethiopia. This disproportionately affected young adults, 55% of prevalent TB cases were under 35 years of age (FMoH, 2011); 39% of the estimated 32, 000 deaths per year wereconcentrated among adults 15 to 64 years of age which affects the more productive portion of the population , the youth, and inturn retards economic growth of the country (Melaku *et al*., 2014).

Currently, Ethiopia is one of the 30 high TB burden countries and also one of the 14 countries with the three lists of the new WHO classification of high burden countries (high TB, high MDR-TB and high TB/HIV burden countries, each consisting 30 countries)(Figure 2). The estimated total TB incidence and HIV-negative TB mortality in Ethiopia in 2016 were 177 and 25/100,000 population, respectively, whch is greater than the global average of 140 and 17/100,000, respectively. All the above facts suggested that TB is stll a major public health security issue in Ethiopia (WHO, 2017).

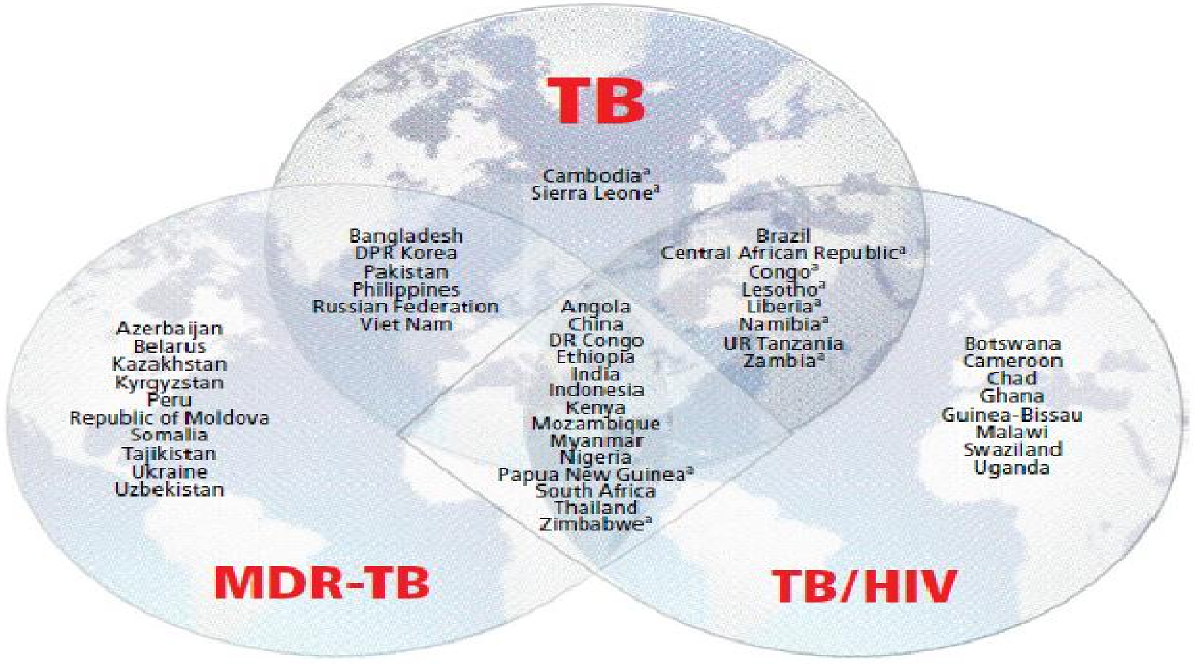

Figure 2.Countries in the three high-burden country lists for TB, TB/HIV and MDR-TB(WHO, 2017). Fourteen countries included in all the three high burden country listsand their areas of overlap: Angola, China, DR Congo, Ethiopia, India, Kenya, Mozambique, Mynamar, Nigeria, Papua New Guinea, South Africa, Thiland and Zimbabwe.

1.4. Global burden of bovine TB

Animal TB is predominantly caused by *M. bovis,* which belongs to the MTBC. TB due to *M. bovis* is referred to as bovine TB, mainly adapted to cattle but also causes TB in other wildlife animal species (Garnier*et al*., 2003) and humans, referred to as zoonotic TB.

In 2016, about 147, 000 new cases of zoonotic TB and 12, 500 deaths were reported due to the disease (WHO, 2017). Among these, the African region consisted the greater number of incidence and deaths due to zoonotic TB, 72, 700 (49.5%) and 9,300 (74.4%), respectively. The South-East Asian region comprized the second greater share, next to Africa, in the number of incident cases and deaths from zoonotic TB which was 46,700 (31.8%) and 2,080 (16.6%), respectively. The Americans and Europe region had the least number of incdent cases, 0.6% and 0.8%, respectively, and deaths due to zoonotic TB, 0.3% and 0.7%, respectively (WHO, 2017).

The transmission of bovine TB at the cattle ï human interface has been long known since the late 1960ôs (Davies, 2006). This demonstrated the cross adaptability of *M. bovis* to different species of hosts suggesting the risk of TB transmission from animals to humans (Davies, 2006). The public health burden of bovine TB has been increasing significantly as humans become more susceptible to *M. bovis* infection (Kleeberg, 1984; Shitaye *et al*., 2007), particularly in developing countries and immunocomprom -ized persons (Radostits *et al*., 2000).

Generally, bovine TB is a neglected tropical disease. Human *M. bovis* infection, for example, is found almost exclusively in low-income countries and minority and migrant populations,originated from these areas, within high-income countries (Cosivi *et al*., 1998; Etter *et al*., 2006; CDC, 2011; Thoen *et al*., 2014). Several studies have detected *M. bovis* and *M. tuberculosis* in rural camel and cattle herds, and in various wildlife species in Africa (Renwick *et al*., 2007; Kudi *et al*., 2012; Moiane *et al*., 2014; Berg *et al*., 2015). It is estimated that in countries where pasteuri zation of milk is rare and bovine TB is common, 10 to 15% human cases of TB are caused by *M. bovis* (Ashford *et al*., 2001). According to some studies from Tanzania, Nigeria and Uganda, *M. bovis* accounted for 20% or more of the MTBC isolated from human TB cases (Mawak *et al*., 2006; Cleaveland *et al*., 2007; Oloya *et al*., 2008).

However, availability of robust data sets and disease surveillance for bovine TB in developing countries is scarce, and is limited to large scale farms and urban health facilities. This may be due to resource limitations as bovine TB surveillance is a logistic and financial intensive work. Moreover, the etiological agent, *M. bovis* is one of the most difficult pathogen to be diagnosed which is exacerbated by the lack of molecular methods as a routine diagnostic tool in such resource limited countries.

1.4.1. Burden of bovine TB in Ethiopia

In Ethiopia, the public health and economic impact of bovine TB has been estimated since its presence is reported in the country (Hailemariam, 1975). Acccording to Tschopp *et al.* (2012), the economic cost of bovine TB has been estimated to be in the range of 75.2 million to 85 million US$ in the rural extensive livestock production and from 500,000ī 4.9 million US$ in the urban livestock production systems between the years 2005ï 2011. Several studies inEthiopia have suggested that bovine TB is prevalent at varying degrees, ranging from <1% to 47%, depending on production system and breed of cattle kept (Ameni*et al*., 2007, 2010; Berg *et al*., 2009; Tschopp *et al*., 2010;Tsegaye *et al*., 2010; Gumi *et al*., 2011; Firdessa *et al.*, 2012).

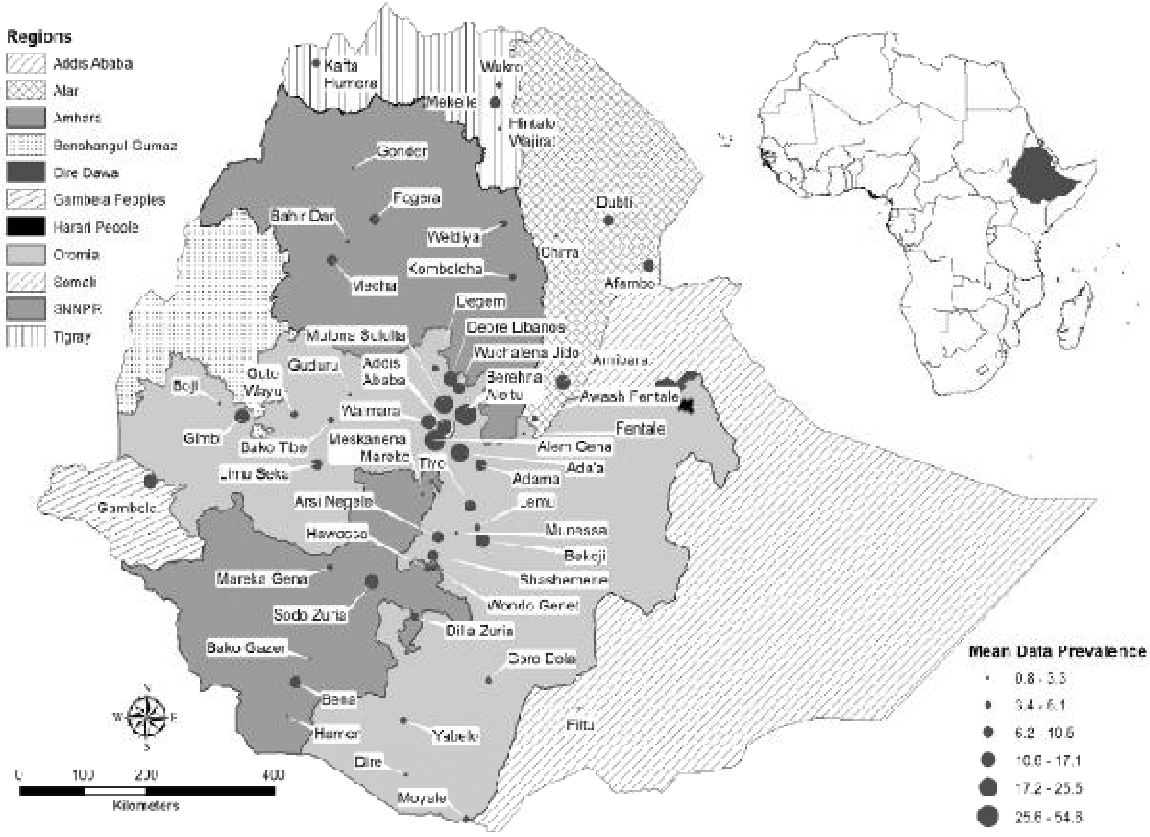

Figure 3. Distribution and mean prevalence of bovine TB (BTB) in districts of Ethiopia (Sibhat *et al*., 2017).

Most of the studies were conducted in Addis Ababa, Amhara, Oromia and Southern Nations and Nationalities Peoples regions where valid published studies were obtained. Variable animal level prevalence of BTB were recorded in the districts of the regions ranging from 0.8% to 54.6%, the highest prevalence being reported in intensive farms in and around cities while the lowest prevalence being recorded in grazing animals in rural areas (Sibhat *et al*., 2017).

Despite the presence of some fragmented studies and minireviews at specific geographical locations, there has not been a national surveillance to have a complete picture on the magnitude of bovine TB in Ethiopia. However, a more recent review in Ethiopia by Sibhat *et al*. (2017) gave a complementary picture in this respect. According to this review, which included findings from research articles published from 2000 to 2016, a pooled prevalence estimate of bovine TB in Ethiopia was found to be 5.8%. The pooled prevalence has been also shown to be affected by both cattle breed type and management system (Sibhat *et al*., 2017). The report suggested that although sufficient data is lacking in some regions of Ethiopia such as Somali and Gambella, valid reports on the distribution of bovine TB are available in all regions of the country (except Benishiangul-Gumuz) (Figure 3).

1.5. Zoonotic transmission of TB

In developed countries, TB caused by *M. bovis* is now rare. This is because of improved socioeconomic conditions and pasteurization of dairy products became extensively practiced (Torgerson and Torgerson, 2008; Michel *et al*., 2010). Nevertheless, an increase in the cases of TB caused by *M. bovis* in some regions of these countries were reported to be due to migrant populations from low and middle resource countries and in most cases, linked to consumption of raw dairy products (LoBue *et al.,* 2003; Rodwell*et al*., 2008).

The lack of diagnostic laboratories with the means to distinguish strains of *M. tuberculosis* and *M. bovis* has contributed to the unknown well defined role of *M. bovis* infection to TB cases in humans in Africa (Ayele *et al*., 2004).

In rural areas of Ethiopia, most people drink unpasteurized milk and live in close proximity to theircattle which intensifies the transmission and spread of BTB. Detection of causal agents of BTB from raw milk (Teshome, 1993; Kiros, 1998) confirms the existing problem and the potential risk of the infection in humans. With respect to this, Kiros (1998) demonstrated that out of human patients with TB, 38.4% were found with extra pulmonary TB(EPTB) and the proportion of patients with EPTB was significant in patients who have close contact with cattle and are in the habit of drinking raw milk in particular. Another study in Ethiopia by Regassa (2012) demonstrated the association of *M. tuberculosis* and *M. bovis* in causing TB between humans and cattle. He confirmed that cattle owned by TB patients had a higher prevalence (24.3%) of contacting TB than those owned by non-tuberculous owners (8.6%). The author also noted that 73.8% and 16.7% of 42 human isolates were identified as *M. tuberculosis* and *M. bovis* and from cattle isolates, 18.1% and 45.5% of 11 were found to be *M. tuberculosis* and *M. bovis* species, respectively.

Although the extent of transmission of *M. bovis* to human beings has not yet been established in Ethiopia, the high prevalence of BTB on dairy farms and the practice of raw milk consumption by the public suggest that the contribution of *M. bovis* on human TB could be important (Ameni *et al*., 2001, 2007; Berg *et al*., 2009).

1.6. The *Mycobacterium tuberculosis*-complex

The Genus *Mycobacterium* consists of phylogenetically grouped complexes such as *M. tuberculosis* complex (MTBC), *M. avium* complex and *M. fortuitum* complex and each complex has its unique characteristics. MTBC species, namely, *M. tuberculosis, M. africanum, M. microti*, *M. canetti,* and *M. bovis* are categorized according to defined number of laboratory phenotypes and genetic markers, but differ in their physiological characteristics, virulence and host range (Niemann *et al*., 2000). In humans, TB is primarily caused by *M. tuberculosis* and *M. africanum* insub-Saharan Africa. In addition, several animal-adapted members of MTBC have been identified. These include, *M. bovis* (cattle), *M. microti* (voles and other small rodents), *M. pinnipedii* (seals and sea lions), and *M. caprae* (goats and sheep).*M. canetti,* witha few strains have been isolated, can cause TB in humans, however, its epidemiological contribution to humanTB is not well defined (van Soolingen *et al*., 1997). Recentlly, *M. mungi,* causing disease outbreak in banded mongoose in Botswana, has been reported as a new member of MTBC (Alexander *et al*., 2010).

1.6.1. *M. tuberculosis* and its genome

Despite the growing public health concern and research progress, the *M. tuberculosis* still holds many secrets in terms of the molecular mechanisms to cause disease and its successful strategy for circumventing host defences, persisting in the host and developing resistance to anti-TB treatment regimens (Chevalie *et al*., 2014).

The organism is a rod-shaped, non-motile, non spore forming, slowly reproducing aerobe. Slow cell division, resistance to detergents and certain antimicrobial agents, persistence in the environment and an acid/alcohol fast nature are due to the characteristic hydrophobic cell wall (Bhatt *et al*., 2007). In contrast to other Gram pos itive bacteria, such as *Staphylococcus* or *Bacillus* species, which have no outer membr -ane, mycobacteria possessa specific a lipid-rich outer membrane, named the mycomembrane, which serves as a similar barrier function to that of the outer membrane of Gram-negative bacteria (Kaur *et al*., 2009). The unique, lipid rich cell wall mainly composed of mycolic acid and peptidoglycan does not take up stain readily, but resists decolourisation when destained with an acid-alcohol wash. This allows mycobacteria to be visualised microscopically using Ziehl- Nielsen stained sputum smears in which the organisms will be seen as red rods embedded in a blue background of the counter stain (Godreuil *et al*., 2007). Mycobacteria are facultative intracellular pathogens infecting host immune cells such as macrophages, where they reside and subsequently disseminate to different parts of the body. The presence of abundant oxygen might be the cause to their great tropism to lung tissues (van Soolingen *et al*., 2001).

The complete, circular genome of *M. tuberculosis* H37Rv was revealed in 1998 (Cole *et al*. 1998), which consisted of about 4.4 million base pairs containing approximately

4000 genes (Figure 4). The genome of *M. tuberculosis* is GC rich (65.6%), a feature that has been associated more often with aerobic prokaryotes (Naya *et al.*, 2002).

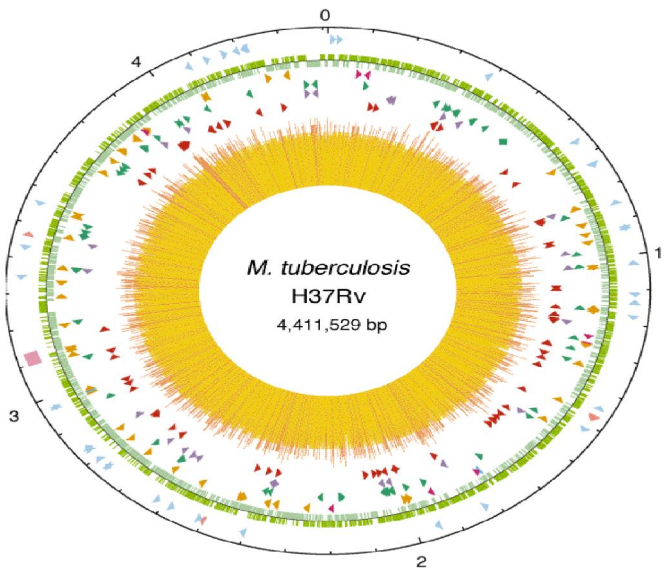

Figure 4.A diagrammatic representation of *M. tuberculosis* H37Rv genome(Cole *et al.,* 1998).

The outer circle shows the scale in megabases, with 0 representing the origin of replication. The first ring from the exterior denotes the positions of stable RNA genes (tRNAs are blue, others are pink) and the direct repeat region (pink cube); the second ring inwards shows the coding sequence by strand (clockwise, dark green; anticlockwise, light green); the third ring depicts repetitive DNA (insertion sequences, orange; 13E12 REP family, dark pink; prophage, blue); the fourth ring shows the positions of the PPE family members (green); the fifth ring shows the PE family members (purple, excluding PGRS); and the sixth ring shows the positions of the PGRSsequences (dark red). The histogram (centre) represents G + C content, with <65% G + C in yellow, and >65% G + C in red (Cole *et al.,* 1998).

The genome of *M. tuberculosis* consisted of genes necessary for the synthesis of most of the nutrients essential for its survial including amino-acids, vitamins and enzyme co-factors (Godreuil *et al.*, 2007). Moreover, *M. tuberculosis* possesses the genes to synthesize glycolytic enzymes and enzymes necessary for the anabolic pentose phosphate pathway, which generates NADPH and pentose sugars, the catabolic Krebś cycle and the glyoxylate cycle, which synthesises carbohydrates from lipids.

The organism is capable of surviving in a number of different environments including the oxygen rich lung, the macrophage and at the centre of caceous granuloma due to the possession of genes coding for enzymes used in aerobic, microaerophilic and anoxic electron transfer (Cole *et al*., 1998).

The *M. tuberculosis* genome is characterized by the presence of a number of repetitive DNA sequences in including insertion sequences (IS), the direct repeat (DR) region, the major polymorphic tandem repeats (MPTR) and the polymorphic GC-rich repetitive sequence (PGRS) (Poulet and Cole, 1995). The abundant repeated DNA sequences are variable and these include duplicate copies of certain genes, insertion elements e.g. IS*6110* found in clusters among several different families and a set of scattered DNA repeats present in several copies e.g., mycobacterial interspersed repetitive units (MIRUs) (Smith, 2003).

1.6.2. *M. bovis* and its genome

Like that of *M. tuberculosis, M. bovis* is a Gram-positive, acid-fast, rod-shaped, aerobic bacteria. However, lack of pyruvate kinase activity in *M. bovis*, due to *pck*A containing a point mutation that affects binding of Mg^{2+} cofactor brings about a slight difference in its energy metabolism to that of *M. tuberculosis*(Garnier *et al*., 2003). Pyruvate kinase catalyses the final step of glycolysis, the dephosphorylation of phosphorenolpyruvate to pyruvate (Garnier *et al*., 2003).

M. bovis has similar pathology to *M. tuberculosis* in humans, causing chronic debilitation, cough, and dissemination to other organs of the body (Garnier *et al*., 2003). Infected cattle suffered from necrotic lesions in the lung and bronchomediastinal lymph nodes (Garnier *et al*., 2003). Moreover, infected cows produce mycobacterial mastitis causing the shedding of the bacteria into milk leading to transmission to humans if the milk is ingested without pasteurization (Garnier *et al*., 2003). *M. bovis* is the ancestor of the most widely used vaccine against TB, *M. bovis* bacillus Calmette-Guérin (BCG) is a strain that was created by growing *M. bovis* on potato slices soaked in ox-bile and glycerol over a period of 13 years (Garnier *et al*., 2003).

Members of the MTBChave been reported to possessinterspecies genetic homogeneity characterized by 99.9% similarity at the nucleotide level and identical 16S rRNA sequences (Sreevatsan *et al*., 1997). This lack of variationmay have resulted from limited selective pressure (Fleischmann *et al*., 2002; Kapur *et al*., 2000).Despite their great genetic relatedness, MTBC members are categorized into different species due to species-specific genotypic variations, such as single nucleotide polymorphisms (SNPs) and deletion of RDs, and phenotypic variations related to host adaptability and virulence (Brosch *et al*., 2002; Galagan, 2014).

The genome sequence of *M. bovis* has been known to be 4,345,492 bp in length, arranged in a single circular chromosome with an average GC content of 65.63% (Figure 5). The genome contains 3,952 genes encodingproteins; including a prophage and 42 IS elements (Table 1). Strikingly, the genome is >99.95% identical at the nucleotidelevel to that of *M. tuberculosis*, showing colinearity and noevidence of extensive translocations, duplications or inversions (Garnier *et al*., 2003).

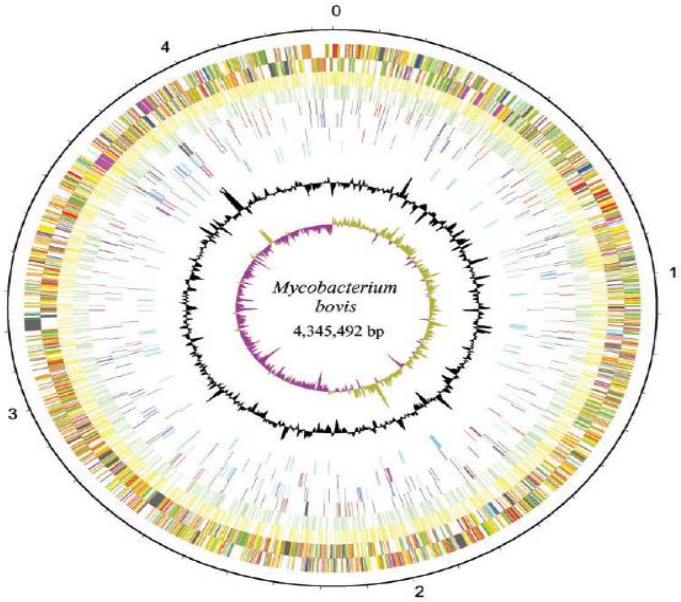

Figure 5. Diagramatic representation of *M. bovis* genome (Garnier *et al*., 2003).
The outer black circles indicated the scale in megabases. From outside to inside, the next two circles show forward and reverse strand coding sequences (CDS), respectivelty, with colors representing the functional classification. Comparisons with the *M. tuberculosis* H37Rv sequence are then shown, with transitions (yellow) and transversions (green), then insertions (red, 1bp; black >1bp) and deletions (dark blue, 1bp; light blue>1bp); sequence replacement by novel regions in *M. bovis* are then shown (purple). IS elements and phage (cyan) are displayed in the following circle, with G+C content and then finally GC bias (G-C)/(G+C) shown by using a 20-kb window(Garnier *et al*., 2003).

In comparison with *M. tuberculosis*, there are 2437 SNPs between them (Table 1). The SNPs have been previously associated with a number of characteristics of *M. bovis*, such as a point mutation on pncA gene which confer anti-TB drug resistance for pyrazinamide (Garnier *et al*., 2003).

Table. 1. Genomic comparison between *M. tuberculosis* and *M. bovis* (Garnier *et al.*, 2003).

Features	***M. bovis* AF2122/97**	***M. tuberculosis* H37Rv**
Genome size, bp	4,345,492	4, 411, 532
G+C, %	65.6	65.6
Protein coding genes.	3,951	3, 995
Compared to *M. bovis*		
SNPs	_	2, 437
Transitions	_	1, 649
Transversions	_	788
Deletions (>1bp)	_	205
Insertions (>1bp)	_	177

1.6.3. Genotyping and phylogenetic classification of *M. tuberculosis* strains

Genotyping of *M. tuberculosis* has an important role to understand the complexity of transmission pathways of the disease it causes within the communities. Hence, it will improve our knowledge of TB epidemiology within a locality. The transmission dynamics of TB has been complex due to the unique behaviour of the pathogen, which infects only a small proportion of individuals and remains dormant for a long period before it springs on, becomes active and transmits infection (Hirsh *et al*., 2004). Understanding transmission pathways using genotyping of *M. tuberculosis* is based on the assumption that patients who are infected with strains exhibiting the same fingerprints or designated to the same clusters are originated from the same recent common progenitor, while those infected with strains having distinct genetic profiles are considered to be unrelated denoting reactivation of infection contracted from another source (van Embden *et al*., 1993; van Soolingen *et al*., 2001).

Several earlier studies reported phylogenetical classification of *M. tuberculosis* complex strains. A sequence diversity in selected genes was described in three principal genetic groups (principal group 1, 2 and 3) which correlated well with spoligotype families (Sreevatsan *et al*., 1997). Brudey and colleagues (2006) grouped 62 spoligotype families or clades from related spoligotypes. From another study, major lineages in the MTBC were defined by identifying large sequence polymorphisms (LSPs) (Gagneux *et al*., 2006). The study also demonstrated strong associations between each of the six characterised lineage groups and geographic region; Indo-Oceanic (IO), East Asian (EAS), East African-Indian (EAI), Euro-American (EA), West African-1 and West African-2.

According to a study by Gagneux and Small (2007) strains can be classified as ancient (also known as ancestral) or modern. The ancient strains, which belong to the principal genetic group 1, include *Mycobacterium africanum* (West African 1 and 2) and *Mycobacterium bovis* as well as the strains forming the Indo-Oceanic lineage (lineage 1). The modern strains include principal genetic group 1 strains belonging to the East Asianlineage (lineage 2) or to the East-African-Indian lineage (lineage 3) and principal genetic group 2/3 strains belonging to the Euro-American lineage (lineage 4).

Nowadays, a set of rules to classify genotypes of the MTBC into major lineages using spoligotypes and MIRU-VNTR results has been formulated (Shabbeer *et al*., 2012). This online tool, TB Lineage, is now freely available at http://tbinsight.cs.rpi.edu/run_tb_lineage.html.

1.7. Genotyping methods used in molecular epidemiology

Genotyping tools have provided better understanding on the transmission pathways, infection source, and spread of mycobacterial strains within a population (Gopaul *et al*., 2006; Mathema *et al*., 2006). Moreover, these methods are used to determine phylogenetic relationships of MTBC strains and provide useful data about epidemiology of TB (Brudey*et al*., 2006). Molecular typing methods are based on the analysis of DNA fingerprints of the *M. tuberculosis* complex and used to identify and differentiat between the strains of MTBC (van Rie *et al.* 1999). Nowadays, there are different genotyping methods that use different genetic elements as strain-specific markers and the most commonly used are described below.

1.7.1. Spoligotyping

Spoligotyping method is based on the DR in the polymorphic MTBC genome which consists of alternating identical DRs of 36 bp and 43 variable spacer DNA sequences of 35-41 bp. This PCR-based reverse hybridization technique exploits the variation in the spacers within the conserved DRs in order to differentiate MTBC strains (Kamerbeek *et al.* 1997). The results are denoted as a 43-digit binary string made based on the presence or absence of spacers (Figure 6).

Spoligotyping is a widely used method as it is simple, rapid, reproducible,cost effective and produces highly diverse portable numerical results (Brudey*et al.,* 2006). Its application on clinical samples allows to process the fingerprinting of large number of samples within very short period of time (Kamerbeek *et al.* 1997). However, spoligotyping is less discriminatory compared to other genotyping methods like-MIRU-VNTR and IS6110 (Soini *et al*., 2001; Oelemann *et al*., 2007).

Spoligotyping differentiates *M. tuberculosis* clinical isolates to a family (subspecies) level (Filliol *et al*., 2002; Sola *et al*., 2003). The patterns of the strains are compared to the recognised reference species patterns stored in the international data base (Brudey *et al*., 2006). The Spoligotyping data could be processed to determine the evolutionary phylogenetic relationship between the strainsalthough some authors argue that this method should not be relied upon alone for evolutionary studies in *M. tuberculosis* (Warren *et al.,* 2002).

Figure 6.Spoligotypes of amplified mycobacterial DNAs.

The hybridization pattern was shown for 35 *M. tuberculosis* and 5 *M. bovis* strains in which the order of the spacers on the filter corresponds to their order in the genome (Kamerbeek *et al*., 1997).

1.7.2. Mycobacterial Interspersed Repetitive Units–Variable-Number Tandem Repeat (MIRU-VNTR)

The genome of MTBC contains variable numbers of tandem repeats (VNTRs) known as the mycobacterial interspersed repetitive units (MIRUs) (Supply *et al.,* 1997). A total of 41MIRU loci were identified when analysing the complete H37Rv genome and the MIRU-VNTR genotyping method differentiates strains based on the polymorphisms in the VNTRs within these loci (Supply *et al*., 2000). The number of repeats identified at 12, 15 or 24 MIRU loci can be used for genotyping using this PCR-based method. Currently, the 24-loci MIRU-VNTR is a reference method due to its high-resolution tool to capture the genetic diversity of *M. tuberculosis* complex strains (Supply *et al.,* 2006; Sloot *et al.*, 2013).

The MIRU-VNTR analyses have advantages in that the results are intrinsically digital and generated data can easily be transported to other centers. As compared to IS*6110* RFLP, only small amounts of genomic material are required as the method involves DNA amplification. The assay is extremely simple to perform when compared with the process involved in carrying out RFLP (Supply *et al*., 2001).

A study by Mathema *et al*., (2006) suggested that the discriminatory power of this method is typically proportional to the number of loci evaluated. However, as some loci are more variable than others, the discriminatory power of MIRU does not increase in a linear manner with the analysis of additional loci.

1.7.3. Restriction Fragment Length Polymorphism (IS6110-RFLP)

The restriction fragment length polymorphism (RFLP) technique is based on the variation in the number and genomic position of the repetitive, mobile insertion sequence (IS) *6110.* TheIS*6110* present in the genomes of the MTBC in varying numbers of copies. The method generates strain specific hybridization patterns (van Embden *et al.,* 1993).

The Molecular fingerprinting using IS*6110* depends on on the cultivation of the organism, genomic DNA extraction, enzymatic restriction, agarose gel electrophoresis, Southern hybridisation and detection of the IS elements with a labelled probe. The method also requires standardization of the restriction enzyme, nature of the labelled probe and the molecular size-markers used (van Embden *et al.,* 1993).

The use IS*6110* RFLP in TB epidemiology for determining the transmission complexity of the disease has several advantages. The method offers good discrimination between unrelated strains, patterns can be digitalized and computerized and can be exchanged between different centers, a standard method and is widely used (Bifani *et al*., 1999). However, the major drawbacks of this method are it is time-consuming and expensive, labour intensive, and requires large quantities of DNA (>200ng). It is also unable to distinguish strains with few (< 5) IS*6110* copies (Gillespie *et al*., 2000).

1.8. Anti-TB drug resstance

1.8.1. Historical Background

Drug-resistant TB become a persistant threatnot long after the first antibiotic was introduced in 1944, drug resistance emerged mainly due to the use of streptomycin as monotherapy(Crofton *et al*., 1948).Until the end of the 1980ŝs, the problem of drug resistant-TB has been given little attention. However, drug resistant TBreceived global attention as a public health threat only in the early 1990s which was coincided with the detection of outbreaks of MDR-TB (defined as resistance to at least RIF, andINH) in the United States, and that were associated with high mortality among patients coinfected with HIV(Frieden*et al*., 1993).

The first inititation towards the surveillance of antimicrobial resistance in the world was conducted in 1994 by the Global TB Program of WHO, with the support of the

International Union against TB and Lung Disease. In the first meeting which was conducted in 1993, researchers from some parts of the world (Dominican Republic, Kenya, and Zimbabwe) took the initiation to implement the first drug-resistance surveys using a standardized approach (Zignol*et al*., 2016).The first global report on anti-TB drug resistance was published by the WHO in 1997 with results from surveys conducted in 35 countries (WHO, 1997). Since then, drug-resistance surveillance data have been published annually in the WHO Global TB Report, allowing rapid sharing of data soon after they become available by incorporating them alongside other aspects of TB surveillance.From2006, the recognition of extensively drug-resistant (XDR) TB (defined as MDR TB plus resistance to a fluoroquinoloneand at least one second-line injectable agent: amikacin, kanamycin, or capreomycin) become an emerging threat world wide (Zignol*et al*., 2016). Currently, all forms of DR-TBbecome major publc health concern in the global TB care and prevention.

The first WHO report on drug resistant TB in Africa was conducted for some countries which had a well-functionng TB control program and asmany countries have not conducted nationwide surveillance of the level of drug resistance. In the WHO two consequative TB-surveyes conducted between 1994 and 1999, only 13 sub-Sahara African countries were included:these were Benin, Botswana, Ivory Coast, Kenya, Lesotho, Sierra Leone,Swaziland, Zimbabwe, Central African Republic, Guinea, Mozambique, South Africa and Uganda. In Ethiopia, following the recognition of the public health importance of TB in the 1960ȇs, starting in the 1970ȇs, separate studies on anti-TB drug resistance were conducted in different parts of the country (Pattyn*et al*., 1979; Lemma *et al*., 1984; Wolde *et al*., 1986; Lemma *et al*., 1989).

1.8.2. Epidemiology of anti-TB drug resistance and associated risk factors

Globally in 2017, the global averages of isoniazid resistance without concurrent rifampicin resistance were 7.1% in new TB cases and 7.9% in previously treated TB cases (WHO, 2018). The same report indicated an estimated 3.5% of new cases and 18% of previously treated cases had MDR/RR-TB at the end of 2017.There were an estimated 558 000 incident cases of MDR/RR-TB in 2017 and the proportion of cases estimated to have MDR-TB was 82% (460 000 out of 560 000) (WHO, 2018).

Drug-resistant TB remains to be a major public health problem in many countries. However, the levels of drug resistance in different countries vary and is generally an indicative of the qualityof TB control and the use of short-course chemotherapy. The most recent data on the global percentage of patients with newly diagnosed MDR-TB remains stable, at 3% or lower, except some Eastern Europe and central Asia countries (Figure 7).

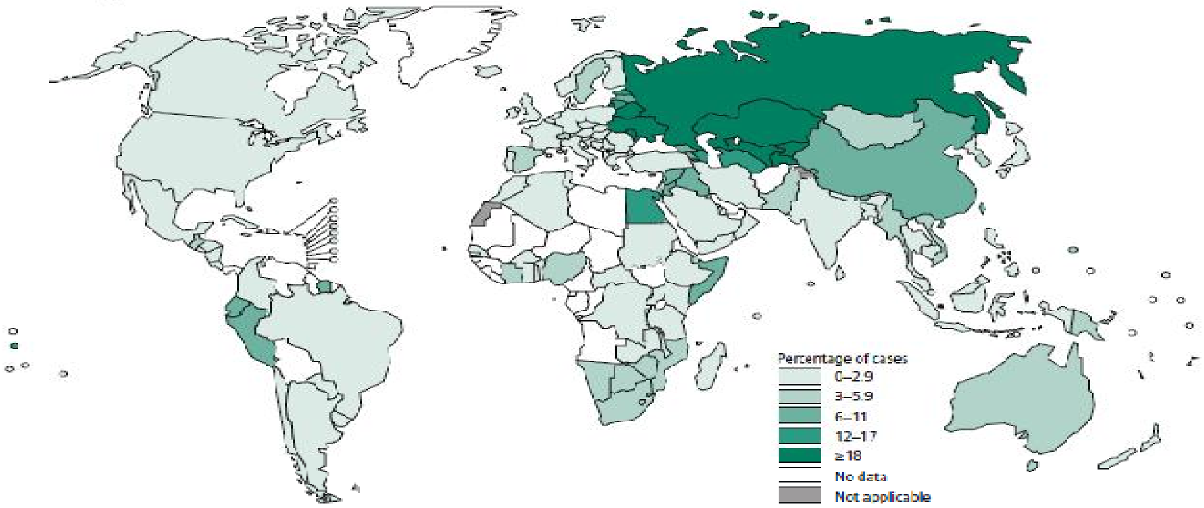

Figure7. Percentage of patients with newly diagnosed TB who have Multidrug/ Rifampicine Resistant (MDR/RR-TB) TB(WHO, 2018).

The figure reflects the most recent year for which data have been reported, which varies among countries. The countries with the largest numbers of MDR/RR-TB cases (47% of the global total) were China, India and the Russian Federation.

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Data from 123 WHO member countries showed the detection of XDR-TB at the end of 2016, the average proportion of MDR-TB cases with XDR-TB was 6.2%, lower than the best estimates of the previous years, 9.5, 9.7 and 9.0% in 2015, 2014 and 2013, respectively. This decrease was explained by the use of a large quantity of routine surveillance data and more precise country-specific measures of the prevalence of second-line drug resistance among MDR/RR-TB cases (WHO, 2017).

Separate studies in different parts of the world indicated the public health threat of all forms drug resistance TB. A cross sectional study conducted in India reported a 40% MDR and 0.2% XDR-TB cases (Myneedu *et al*., 2015). About 40.95% cases were resistant to atleast one of the first-line anti-TB drugs (28.73% INH-monoresistance, 19.41% RIF-mono resostance) and 16.6% of them were with MDR-TB (Shao *et al.,* 2011). A lower (6.3%) levels of INH monoresistance cases was detected in a study conducted in Pakistane (Ayaz *et al*., 2012).

Resistance to one of the first-line anti-TB drugs were reported in some East and other parts of African countries such as kenya(30%) Tanzania (11.4%) Rwanda(43%) and Uganda (10.3%) (Gafirita *et al*., 2012; Ndungũ *et al*., 2012; Lukoye *et al*., 2013; Hoza *et al*., 2015). Studies from other parts of Africa such as Zambia, Zimbabwe and SouthAfrica reported significant proportions of *M. tuberculosis* isolates that were resistant to at least one of the first-line anti-TB drugs (Silaigwana *et al*., 2012; Sagonda *et al*., 2014;Kapata *et al*., 2015). The source of variations in the frequencies of resistance among TB cases may be due to differences in patients categories as new and previously treated, adherance to anti-TB drugs, loss to follow up of under treatment cases, health care access or the effectivness of the over all TB control strategies at various locations.

Studies conducted from different parts of Ethiopia from 1978 to 2005 showed that the primary resistance to INH ranges from 1.9% to 21.4% and for RIF from 0% to 1.9%, and MDR-TB ranges from 0% to 1.3% (Gebremedhine*et al*., 2014). A previous study from Addis Ababa, Ethiopia, reported a higher (72.9%) resistance to at least one of the first-line anti-TB drugs (Abate *et al*., 2012).Similar studies from Oromia region andsouthwest Ethiopia, showed a 33% and 27.7% laboratory confirmed MDR-TB cases, respectively (Mulisa *et al*., 2015; Tadesse *et al*., 2016). However, no MDR-TB cases were detected in a study conducted at Gondar, northwest Ethiopia (Alemayehu *et al*., 2014).

In the Amhara region of Ethiopia, several separate health-facility based studies have been conductd to assess the magnitude of the anti-TB drug resistance in small sections of the population. A previous study in the region revealed a higher (76.9%) drug resistance level from newly diagnosed TB cases and a comparable (23.1%) resistance for retreated cases (Biadglegne *et al*., 2013). Similarly, a study in Amhara region (Dessie) of Ethiopia showed the absence of RIF monoresistance in neither of HIV-positve nor negative TB patients (Maru *et al*., 2015).Higher perecentages of MDR-TB cases, 18.46% (Esmael *et al*., 2014) and 46%, (Maru *et al*., 2015) from retreatment cases has been reported in the region, however, another study in the other part of the Amhara region, Gondar, revealed no resistant mycobacterial isolates for first line anti-TB drugs except one isolate for streptomycine (Alemayehu *et al*., 2014).

Currently, Ethiopia is among the 30 high MDR-TB countries in the world (Figure 2). At the end of 2016,.there were an estimated 2, 900MDR/RR-TB cases from whichabout 2.7% and 14% TB cases were new and previously treated cases, respectively (WHO, 2017).

Drug resistance may ariseas a result of a lack of standardized treatment regimens, poor implementation of theregimens, shortages of drugs, and use of drugs of questionable quality. Other factors include failure to monitor the patientsô treatment and non-adherence on the part of thepatients. In particular, the level of multidrug resistance can serve as a measure of the performance of the national TB program in the country (WHO, 2017).

Increased pattern of anti-TB drug resistance in many parts of the world, notably in high burden African countries including Ethiopia, urges timely surveillance and identification of the potential risk factors for better intervention on the disease. Several studies associated patient and clinical characteristics such as previous treatment cases, age,sex, occupation, previous history of contact to TB patients and forms of TB as predictors for development of anti-TB drug resistance (Bazira *et al*., 2011; Abate *et al*., 2012; Biadglegne *et al*., 2014; Seyoum *et al*., 2014;Mulisa *et al*., 2015;). Considerable number of previous and recentstudies elsewhere in the world associated genotypes of *M. tuberculosis* with development of drug resistance (Kibiki *et al*., 2007; Bazira *et al*., 2011; Ayaz *et al*., 2012; Bedewi *et al*., 2016; Brhane *et al*., 2017; Mathuria *et al*., 2017).

Currently,a 6-month regimen of four first-line drugs: isoniazid (INH), rifampicin (RIF), ethambutol (EMB) and pyrazinamide (PZA) is recommended treatment for cases of drug-susceptible TB. This regimen consists of a 2-month óintensiveô phase, where INH, RIF, PZA and EMB are administered, followed by a 4-month ócontinuationô phase with only INH and RIF (WHO, 2017). Treatment for RR-TB and MDR-TB requires a longer regimens (9ï 12 months) more expensive and toxic drugs. Although the Bacilli Calmette-Guerin (BCG) vaccine, which was developed a century ago is still widely used and has been shown to prevent severe forms of TB in children, there is currently no effective vaccine for preventing TB disease in adults. Currently, there are 12 TB vaccines in Phase I, Phase II or Phase III trials (WHO, 2017).

1.8.3. Molecular basis of anti-TB drug resistance

Understanding the molecular basis of drug resistance is essential to the development of new drugs and provides useful insights for managing patients with drug resistant-TB. Advancements in the development of molecular biology toolsgive insights to the new information on the complete genome sequence of *M. tuberculosis,* which inturn increased our understanding of the mechanismsof anti-TB drugs resistance (Telenti, 1997).

MTBC develops drug resistance as a result of spontaneous mutations in genes encoded on the chromosome (Figure 8), producing the selection of resistant strains during ineffective patient treatment (Zhang and Yew, 2009), unlike the condition in some other bacteria in which acquired drug resistance is generally mediated through horizontal transfer by mobile genetic elements, such as plasmids, transposons or integrons.These mutations in *M. tuberculosis* include single nucleotide changes, small insertions and deletions or larger deletions, and either modify the drug target itself, silence drug activating enzymes in the case of pro-drugs, or circumvent drug action by increasing the gene product targeted by the drug (De Rossi*et al.*, 2006).

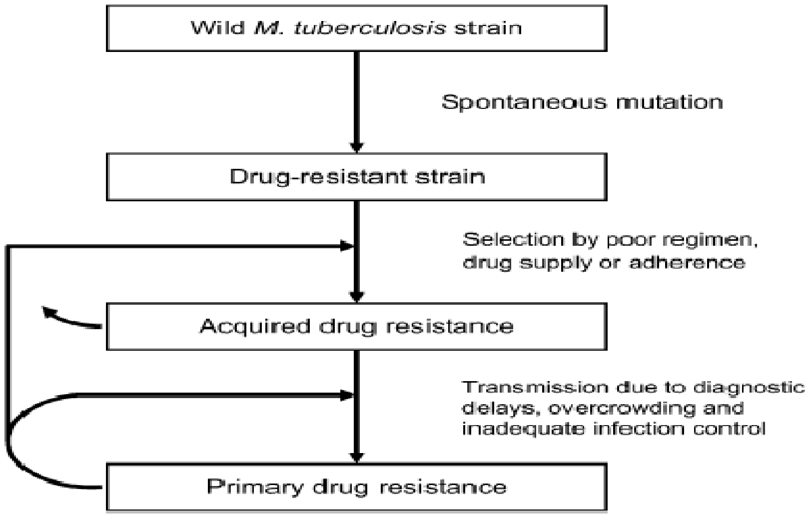


Figure 8. Concepts in the development of drug resistant TB (Zhang and Yew, 2009).

The drug sensitive wild *M. tuberculosis* strain undergoes spontaneous mutations and become drug resistant. The primary anti-TB drug resistance could be acquired through diagnostic delays, overcrowding or inadequate infection control.

The probability of acquiring resistance through spontaneous mutations varies by drug,ranging from approximately 3.1×10^{-8}bacilli for RIF, andto approximately 3.5×10^{-6} bacilli for INH, streptomycin (STR) and EMB (Maruya, 2011). Hence, delivering a combination of anti-TB drugs decreases the risk of acquiring resistance mutations, as the risk of a mutant containing two resistance mutations is $<10^{-18}$ (Gillespie,2007). Moreover, *M. tuberculosis* comprises various phylogenetically distinct lineages (Gagneux*et al*., 2006), and recent studies indicate that the rate of mutation towards drug resistance might be influenced by the lineage to which a particularstrain belongs (Ali *et al*., 2016; Bedewi *et al*., 2017).

1.8.3.1. Resistance to first line anti-TB drugs

Currently, the recommended treatment for cases of drug-susceptible TB is a 6-month regimen of four first-line drugs: INH, RIF, EMB and PZA (WHO, 2017). Previous studies suggested the link between resistance to first line anti-TB drugs and mutations in at least 10 genes; *katG*, *inhA*, *ahpC*, *kasA* and *ndh* for INH resistance; *rpoB* for RIFresistance, *embB* for EMB resistance, *pncA* for PZA resistance and *rpsL* and *rrs* for STRresistance (Maruya, 2011).

Isoniazid (INH): The anti-TB activity of INH was introduced in the early1950s and INH-resistant clinical isolates frequently lost catalase and peroxidase activity (Middlebrook, 1954). The catalase-peroxidase was knownfor its activation of INH to a toxic substance which subsequently affects intracellular targets such as mycolic acid biosynthesis (Zhang, 2005).However, until the early 1990s, no direct evidence was obtained for the association of this enzyme with INH activation when the primary mycobacterial catalase-peroxidase gene (*katG*) was cloned and sequenced. Consquently, a study conducted in Italy also identified *katG*, *ahpC*, *inhA* promoter and *kasA* genes as responsible genes for INH resistance (Zhang, 2005).

The sequencing the *inhA* promoter region, the *inhA* gene, and the *katG* genecan detect about 70-90% of INH-resistant isolates.INH resistance can be attributed to mutations in the *inhA* promoter region which leads to over production of the drug target.Low-level INHresistance canfrequently occur due to mutations in the *inhA* gene promoter and which are present in 10ï 20% of resistant strains (Miotto*et al*., 2008).

A study from Saudi Arebia revealed high frequency of *katG* 315 mutations (65.2%) in INH resistant strains were found and were predominant among the newly diagnosed cases (Varghese *et al*., 2012).

Recent studies from China and Ghana reported higher frequencies of *katG* mutations among INH-resistant isolates, 75.8% and 92.5%, respectively (Otchere *et al*., 2016; Daoqun *et al*., 2017). Higher frequencies of *katG*315 mutations were also reported in Pakistan, 82% and Russia, 93% (Ali *et al*., 2011). A study in Amhara region, northern part of Ethiopia reported that all the INH-resistant isolates were due to mutations at *katG* gene (Biadglegne*et al*., 2013). Higher frequencies of *katG* mutations were also reported in other parts of Ethiopia (Bedewi *et al*., 2016; Tadesse*et al*., 2016; Brhane*et al*., 2017).

Rifampicin (RIF): The significant early effect on metabolic activity of *M. tuberculosis* and excellent late sterilizing action on semi-dormant organisms, under going short bursts of metabolic activity, let RIF to be introduced as an effective anti-TB drug in the early 1970ǿs (Somoskovi*et al*., 2001; Zhang, 2005). Unlike that of INH, monoresistance to RIF is rare. Instead, nearly 90% RIF resistance occurs in strains that are also resistant to INH; thus, RIF resistance can be used as a marker for MDR-TB (Zhang, 2005).

RIF acts by inhibiting mycobacterial transcription by targeting DNA-dependent RNA polymerase. The resistance to RIF is due to mutations in a well-defined, 81 base pair (bp) (codon 507 to 533) central region of the gene,known as the RIF resistance determining region (RRDR) that encodes the β-subunit of RNA polymerase (*rpoB*). Mutations in this region affect the protein structure of the target so that RIF can not bind; thus, conferring resistance. More than 96% of the RIF-resistant *M. tuberculosis* strains contain a mutation in this specific gene zone, hence, essential for a rapid detection of RR/MDR-TB (Zhang and Telenti, 2000; Zhang, 2005).

A study from Pakistan reported the detection of mutations at four amino acid codons of *rpoB*, codons 531 (68%), 516 (24%), 526(4%), and 513 (2%) in 49 of 50 isolates (Ali*et al*., 2011). Another study from China reported common mutations at codons 526 and 531, and mutations at S531L was found in 14 of the17 RIF-resistant strains (Daoqun*et al*., 2017). From a previous study conducted in Ethiopia, about 80% of the total RIF-resistant specimens were due to amino acids changes at *Ser531Lue* codon of the *rpoB* gene sequence (Brhane*et al*.,2017). Many other studies in Ethiopia also suggested a higer frequencies of *Ser531Lue* codon mutations, however, an over all low level of RIF-mono resistance has been noticed in the country (Seyoum *et al*., 2014; Hamusse *et al*., 2016; Bedewi *et al*., 2017).

Pyrazinamide (PZA)**:** PZAwasintroduced into TB chemotherapy in the early 1950s (Silva and Palomino, 2011). Since then,it has become an important first-line anti-TB drug that is used inshort-course chemotherapy and is one of the cornerstone drugs in the treatment of MDR-TB (Mitchison, 1985). Its use, along with other first lne anti-TB drugs, allowed the length of treatment to be reduced from 9 to 6 months. PZA has an important characteristic of inhibiting semi dormant bacilli residing in acidic enviro -nments**.** PZA is apro drug that needs to be converted into its active form, pyrazinoica cid (POA), by the enzyme pyrazinamidase/nicotinamidase (PZase).

PZase is encoded in *M. tuberculosis* by the gene *pncA* (Scorpio and Zhang, 1996) and mutations in *pncA* are the main mechanisms for PZA resistance in *M. tuberculosis*. Most of these changes occur in a 561 bp region (of the open reading frame) or in an 82 bp region (of its putative promoter) (Jure´en *et al*., 2008). Although *pncA* gene mutation is well established among PZA-resistant *M. tuberculosis* strains, some PZA-resistant strains do not show mutations in *pncA* or its promoter region which might be because of mutations occurring in an unknown *pncA* regulatory gene (Cheng*et al*., 2000).

Recently, a study conducted in Veitnam reported that many different *pncA* mutations (71 types) were detected highlighting the high rate of PZA resistance-associated mutations in*M. tuberculosis* isolates (Huy*et al*., 2017). Findings from the whole-genome sequence analysis has identified mutations in the *panD* gene encoding aspartate decarboxylase in PZA-resistant strains lacking *pncA* and *rpsA* mutations, suggesting that *PanD* represents a new target of PZA/POA (Shi*et al*., 2014). However, another recent study proposed that PZA resistance was independent of mutations in *panD* but molecular heterogeneity in *pncA* was associated with resistance and should be evaluated as a diagnostic tool (Ramirez-Busby*et al.,* 2017).

Ethambutol (EMB): EMB [dextro-2,2ô-(ethylenediimino) di-1-butanol] was first introduced as an anti-TB drug in 1966. EMB is an essential first-linedrug in tuberculosis treatment, together with INH,RIF and PZA, the other first-line drugs currently in use. EMB is active against multiplying bacilli where it targets the mycoba cterial cell wall through interaction with arabinosyl transferases involved in arabinoga lacton (AG) and lipoarabinomannan (LAM) biosynthesis. It specifically inhibits polymerization of cell-wall arabinan, thereby leading to accumulationof b-D-arabinofuranosyl-1-monophosphoryl decaprenol (DPA) (Takayama *et al*., 1979; Barco*et al*., 2006).

In *M. tuberculosis*, the genes *emb*CAB were arranged as a 10 kbp operon encodingfor mycobacterial arabinosyl transferase, an enzyme involved in the synthesis of arabinogalactan (Telenti *et al*., 1997). Mutations in the *embCAB* operon, in particular *embB*, and occasionally *embC*, are responsible for resistance to EMB.Point mutations of codon 306 in *M. tuberculosis* gene *embB* have been shown to be the most frequent and indicator mutations for EMB resistance.

However, point mutations in the *embB306* locus occur in only about 60% of all EMB-resistant isolates (Ramaswamy *et al*., 2000; Lee *et al*., 2004), and *embB306* mutations can also occur in EMB-susceptible isolates (Mokrousov *et al*., 2002)., which may suggest for the presence of other mechanisms of EMB resistance.

A study in China detected 29 *embB* mutated types among 101 isolates tested, including 74 (93.7%) EMB-resistant isolates and 27 (45.0%)EMB-susceptible isolates (Zhao *et al*., 2015). According to this study, the majority of *embB* mutations were observed in the region between codons 306 and 497 and mutations in this region showed strong correlations with EMB resistance. Among 23 EMB-resistant isolates, nucleotide sequencing showed that 16 strains had a mutation in the *embB*gene andthe most common mutation observed in *the embB* gene was at codon 306 (Srivastava*et al*., 2006).

1.9. Global Control of TB

1.9.1. Human TBControl

The history of TB control dates back to the time of attempts of compulsory treatment of unidentified cases through vertical approach (Frieden *et al*., 2000; Raviglione, 2002). In developed nations, the annual TB infection rate was reduced by 5-10% after the introduction of effective treatment of the identified cases. However, similar results were not obtained in developing countries due to limitations of resources and poor general living conditions (Mahler, 2007). This was also complicated by the HIV epidemic in these areas (Miller, 2000).

In response to the global high public health burden of TB and affordability of anti-TB drugs, the WHO declared a global emergency in 1993 and promoted a TB management strategy called directly observed therapy short course (DOTS). About 50 million TB patients were cured using the DOTS between the years 1995-2008, and this prevented up to 7 million deaths (Marais, 2013). The WHO identified 5 key components of the strategy:

- Government commitment to ensure lasting and comprehensive TB control
- Case detection by sputum smear microscopy among self-reporting symptomatic patients.
- Standardized short course chemotherapy using 6-8 months treatment regimen.
- Regular and uninterrupted supply of anti-TB drugs.
- Standardized recording and reporting system.

The DOT strategy was believed to ensure proper medication (right dose and time interval of drug administration) and increase case detection rate (CDR) and treatment. However, the effectiveness of the strategy was limited in high burden and resource poor settings (Fochsen *et al*., 2009). Lack of privacy and stigmatization during DOT are also raised as ethical concerns (Selgelid and Reichman, 2011).

To improve efforts of TB control, the WHO designed different strategies. The Stop TB partnership, which was begun in 2006, widened the global effort of TB control.

The .strategy aimed to address management of all forms of TB including HIV-associated and DR-TB, through engagement of communities, involvement of all care providers,strengthening of health systems, and fostering of research. Subsequently, between 2000 and 2013; about 37 million lives were prevented and a new rapid molecular test to simultaneously diagnose TB and RIF resistance was developed (Uplekar *et al*., 2015).

For the period 2016ï 2035, the WHO targeted an End TB Strategy which is inline with the United Nationsô (UN) Sustainable Development Goals (SDGs) to end the global TB epidemic. The global targets set in the End TB Strategy include a 90% reduction in TB deaths and an 80% reduction in TB incidence by 2030, compared with 2015. The first targetes of the End TB Strategy are set for 2020. They are a 35% reduction in TB deaths and a 20% reductionin TB incidence, compared with levels in 2015; and that no TB patients and their households should face disastrous costs as a result of TB disease (WHO, 2014).

According to the WHO (2018) report, currently, three major categories of health interventions are available for TB prevention:

- Treatment of latent TB infection (LTBI)
- Prevention of transmission of *M. tuberculosis* through infection prevention and control
- Vaccination of children with the bacille Calmette- Guérin (BCG) vaccine.

Although significant progress of TB control was shown over the last two decades, the disease remained to be one of the worldôs health threats, particularly in developing countries (Li *et al*., 2017).

1.9.1.1. TB control in Ethiopia

The history of TBcontrol programme in Ethiopia goes back to the early 1960s,half a century ago.The effort to control tuberculosis began with the establishment of TB centers and sanatoria in three major urban areas in the country, namely: Addis Ababa, Asmara, and Harar. In 1992, following the start of the national TB and leprosy control program (NTLCP), a regular TB prevention and control programme was implemented as a pilot in some zones (Arsi and Bale) of Oromia Region. Since 1995, the country adopted and scaled up the WHO recommended DOTS strategy (FMoH, 2010). In Ethiopia, nearly 92% of hospitals and 95% of health centres implemented DOTS-based services in 2011 (FMoH, 2007, 2008).

In the past 20 years, about 1·5 million cases of TB were notified and treated in Ethiopia. Subsquently, a reduction in maortality (by 63%) and prevalence (by more than 50%) has been registered since 1990 (worldwide, mortality fell by 45% and prevalence by 41% during the same period (Burki, 2015).

Despite the progress made in TB control, currently, Ethiopia is one of the 30 high TB burden countries in the world. In 2017, the estimated annual incidence rate (including the HIV + TB) of TB was 164 per 100, 000 population (WHO, 2018). Hence, to understand the present magnitude and complexity of TB problem, the country has to revise the national TB strategic plan and set more concrete targets.

1.9.2. Animal TB control

Globally, the public health burden of zoonotic TB is threatening. According to the WHO (2016) report, there were 147,000 new cases of zoonotic TB in people leading to 12,500 lost lives due to the infection. During 2015 to 2016, more than half of 179 countries and territories reported the presence of the disease in livestock and/or wildlife, demonstrating its wide geographical spread (WHO, 2017). Bovine TB becomes a public health concern to communities that rely on livestock for their livelihoods. The disease has an important economic impact through reduced meat and milk production. Bovine TB also poses a problem to the global commerce of animals and their products (WHO, 2017).

In 2016ï 2017, a roadmap for zoonotic TB control was launched by the union of three organizations: WHO, the World Organisation for Animal Health (OIE) and the Food and Agricultural Organization of the United Nations (FAO), together with the International Union against Tuberculosis and Lung Disease. The roadmap calls for a multidisciplinary ñOne Healthò approach that includes a more comprehensive analysis of risks, better coverage of interventions, more efficient use of resources, reduced costs and ultimately, improved health of human and animal populations (WHO, 2017). The road map has identified 10 priority areas for tackling zoonotic TB in people and bovine TB in animals. These fall under three core themes:

- Improving the scientific evidence base
- Reducing transmission at the animal-human intersection, and
- Enhancing intersectoral and collaborative approaches.

Although zoonotic TB remains a global problem, the condition is more frequent in developing countries (Ayele *et al*., 2004). However, most of the disease control strategies developed failed to consider the socio-cultural practices and habits of most at risk populations (Carruth *et al*., 2016).

Despite the efforts made in human case identification and treatment of zoonotic TB, there has been a general lack of focus in controlling the disease. The training package of the community health extension program in Ethiopia lacks a discipline related with prevention and control of diseases transmitted from animals and their products to human (FMOH, 2005). The control of zoonotic diseases is labour and cost intensive. Hence, it is better to prevent the diseases using community based intervention programs such as prevention package, awareness creation programs and controlling the origin, source and vehicles of transmission of the disease (Hadush, 2016).

1.10. Rationale of the study

Understanding the epidemiology of TB and initiating appropriate therapy are important for an effective control of the disease. In addition, information on drug susceptibility pattern of the bacterial strains is essential in designing a quality preventive and treatment strategies. Such studies are also important indicators for the spread and recent transmission of the disease within a community. Understanding the possible association between drug resistance pattern and genotypes of mycobacterial strains is also vital to detect if drug resistance is influenced by the genetic and evolutionary background of the bacterial strains. Aditionally, data on TB transmission within the human-animal interface is important in designing a ớOne Healthô approach in the control of the disease, particularly, in countries such as Ethiopia where the livelihood of the majority in rural settings depends on their livestock.However, lack of diagnostic tools with the means to distinguish strains of *M. tuberculosis* and *M. bovis* and the asymptomatic clinical manifestations in the latter has contributed to the unknown well defined role of *M. bovis* infectionin human TB in low-income countries such as Ethiopia.

In the Amhara region, northwest Ethiopia, information on the public health risk of zoonotic TB is scarce.Few cross sectional studies focused on assessing animal prevalence of BTB documented a 3.55% and 8.7% individual cattle apparent prevalence (Mengistu *et al*., 2015; Nuru *et al*., 2015). Isolation of *M. bovis* , causative agent of bovine TB, in humans (Nuru *et al*., 2017) and the observed higher prevalence of tuberculin reactor cattle owned by active TB individuals than those of TB free ones (Mengistu *et al*., 2015) suggested the potential risk of zoonotic transmission of TB between human and their associated cattle in the region.

However, there is scarcity ofdata on human and animal TB in South Gondar Zone of the Region, where the lives of the vast majority of the population depends on subsistence smallholder dairy farming and well known indigenous population of milk cattle (such as the Fogera cattle) are present. Hence, the magnitude of TB transmission from animals to humans is not well known. The local population does have a long time tradition of consuming raw and locally prepared diary products, and raw meat. In addition, the tradition of slaughtering cattle at the backyard with minimal protection may contribute to zoonotic transmission of TB infection in the area.

In the Region, along with the risk of zoonotic transmission, the emergence of drug resistant TB in humans and the unknown transmission dynamics of the disease and diversity of *M. tuberculosis* strains hamper the effort in the control of the disease. Few studies have been conducted in the Region in this respect (Wondimeneh*et al.,* 2012; Amare *et al*., 2013; Addis *et al*., 2015;Maru*et al*., 2015; Gebrecherkos*et al*., 2016). However, most of such separate studies were health institution based targeting the local urban residents and patients at specific settings such as public prisons. Such studies, however, did not provide enough information aboutthe burden of all forms of TB in the general population includingthe marginalized patients from rural and semi-urban areas. To our knowledge, in South Gondar Zone, epidemiological information on TB both in humans and cattle is unknown.

Therefore, the aim of this epidemiological study is to determine the overall prevalence of TB and its zoonotic transmission between farmers and their cattle, determine the genetic diversity and drug sensitivity pattern of mycobacteria circulating and causing TB in South Gondar Zone, northwest Ethiopia. Thus, results of this epidemiological study will help in strengthening TB control strategies in the country as a whole and in the study area in particular.

1.11. Hypothesis of the study

The prevalenceof TB in farmers and their cattle is high in smallholder dairy farming system in South Gondar Zone and the strains of *M. tuberculosis* isolates will be genetically diverse with a relatively high resistanceto the first line anti-TB drugs.

1.12. Objectives of the study

1.12.1. General objective

The general objective of this study is to investigate the epidemiology of TB in farmers and their cattle, and evaluate the drug sensistivity of *M. tuberculosis*in South Gondar Zone, northwest Ethiopia.

1.12.2. Specific objectives

1. To determine the prevalence of human TB in individuals visiting health facilities in South Gondar Zone, northwest Ethiopia.

2. To identify the *M. tuberculosis* strains and lineages circulating in South Gondar Zone, northwest Ethiopia.
3. To evaluate the drug sensitivity of *M. tuberculosis* isolates to the first line anti-TB drugs in South Gondar Zone, northwest Ethiopia.
4. To determine the prevalence of TB in cattle owned by TB-positive and -negative households in South Gondar Zone, northwest Ethiopia.
5. To confirm zoonotic transmission of TB between humans and their cattle in the small holder dairy farming system in South Gondar Zone, northwest Ethiopia.

2. MATERIALS AND METHODS

2.1. Study site, sampling population, sample size, study design and flow

2.1.1. Study site

The study was conducted from March 2015 to April 2018 in South Gondar Zone of the Amhara Region, North-western Ethiopia (Figure 9). Debre Tabor is the seat of the administration of the Zone and is located at 666 km from Addis Ababa in the northwest direction and at 99 km from Bahir Dar City in the same direction. The Zone consist of 10 districts and inhibited by 2,051,738 people. However,for convenience, data from the Lay Gayint (the upper Gayint) and the Tach Gayint (the bottom Gayint) districts were simply merged as Gayint. Similarly, the data of the Mirab Este (West Este) and Misraq Este (East Este) districts were taken as Este.

The population density of the Zone is 145.56 per square km; 90.47% of its population live in the rural area. The total number of households in the Zone were 468,238 and average number of persons per a household was 4.38 (CSA, 2007). The livelihood of the community largely depends on subsistence agriculture (EDHS, 2011).

The zone is known for its diverse topography ranging from flat and low grazing land to high cold mountains. The majority of the cattle population in the study area is indegineous Zebu cattle maintained in rural areas under extensive husbandary system. The region is known for its indigenous milk producing cattle breedssuch as the ǒFogeraǒ cattle. Dairying is commonly practiced using small herd size. Cases of TB patients who are admitted to the health centers are increasing each year. However, the epidemiology and degree of zoonotic transmission of tuberculosis from animals to humans or vice versa is unknown.

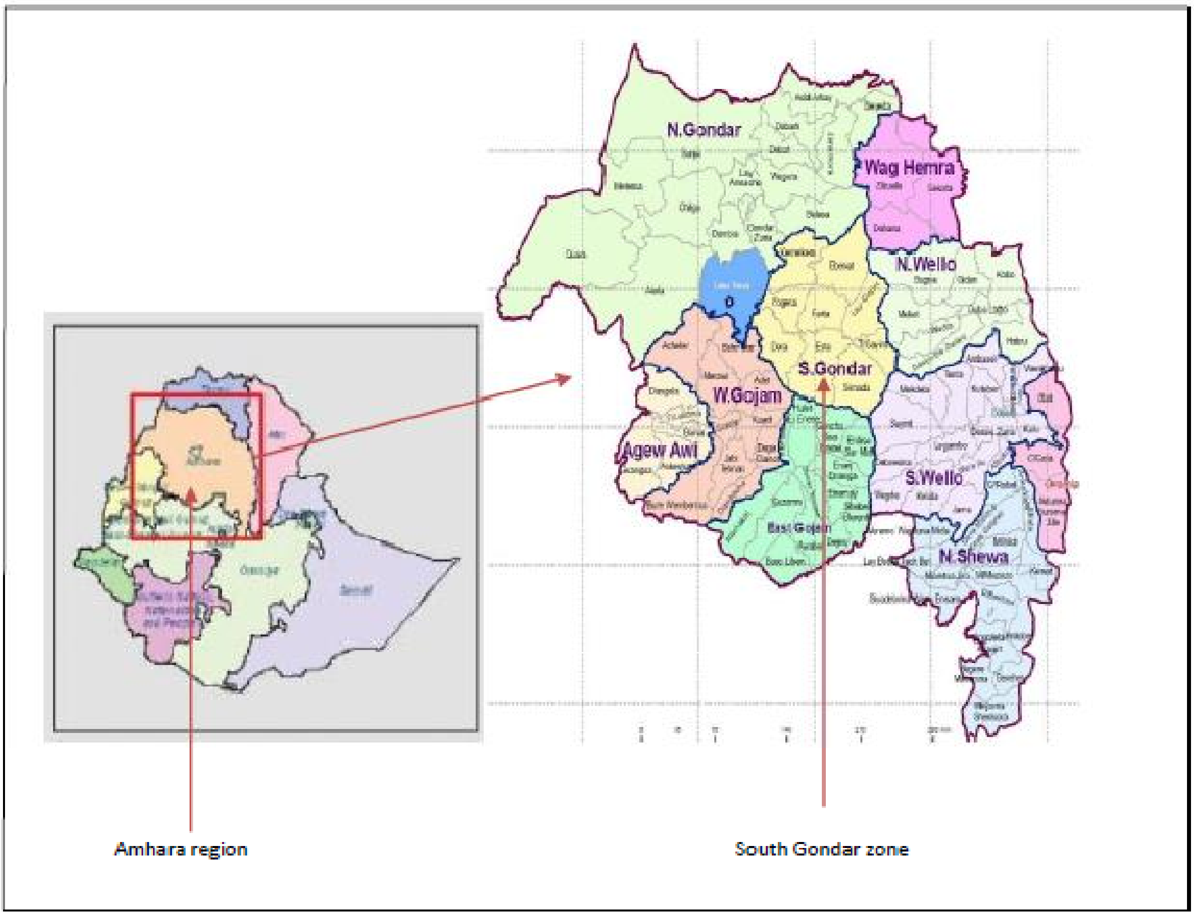


Figure 9. Location map of south Gondar zone of Amhara region, northwest Ethiopia (Source: Modified from Annika, 2016 and Awulachew *et al*., 2009).The South Gondar Zone is located at the Central part of the Amhara region (yellow)and bordered on the south by East Gojam, on the southwest by West Gojam, on the west by Lake Tana (blue), on the north by North Gondar, on the northeast by Wag-Himra, on the east byNorth Wollo, and on the southeast bySouth Wollo zones. The Zone is divided into 10 administrative districts (Dera, Ebinat, East Este, Farta, Fogera, Libo-Kemkem, Lower Gayint, Simada Upper Gayint and West Este).

2.1.2. Sampling population

Human cases were recruited from the different health facilities (rural and peripheral health posts, district health centers, government/private referal hospitals).Active tuberculosis patients (AFB positives) who visited the health facilities seeking TB-treatment were included. The control human subjects were those TB-free(AFB negatives) individuals from the same population which gave rise to the cases. Cattle which were kept at different villages in the district and fulfilling the inclusion criteria for the study, were also our sample sources.

2.1.3. General inclusion and exclusion criteria

Smallholder dairy farmers who are permanently living in the zone for at least two months prior to the study, aged five years and above at the time of the study as well as who consented and willing to participate in the study were included. Whereas, individuals who were not willing to participate aged below five years and seriously ill, were excluded from participating in the study. All cattle owned by human cases and controls older than six months within a herd, except clinically sick ones and cows one month pre-and post partum, were tested by single intradermal comparative tuberculin test (SICCTT).

2.1.4. Study design andSample size determination

1. A cross sectional study, which estimates the prevalence of TBin humans and tuberculin reactor cattle along with the associated risk factors, was conducted. The genetic diversity and drug resistance patterns of mycobacteria circulating and causing TB in the study area were also assessed.
2. A case-control study was conducted to determine the zoonotic transmission of TB among humans and their cattle. Human subjects diagnosed with active TB were traced back to determine tuberculin reactivity of their cattle.

For the cross-sectional human TB prevalence study, all human TB suspected individuals who visited the health facilities in the study area within the data collection period (2015-2017) were recruited. Accordingly, from the eight study districts in the Zone, a total sample size of 3168 was determined.

The case control study for the zoonotic transmission of TB between human TB patients and their cattle was a very labour, time and financial intensive work. Hence, we had limitations to trace back all the TB suspected individuals which were geographically highly diverse within eight districts of the Zone. Therefore, we only managed to reach out to 63 TB positive and 32 TB negative neighbouring households. Based on this, a total of 476 cattle (315 from TB positive households and 161 from TB free households) were tested for bovine TB.

2.2. Data collection and processing

2.2.1. Questionnaire survey

After eligible subjects were recruited and consented (Appendix I) to the study, socio-demographic data were collected using a structured questionnaire (Appendix II). Data on patient characteristics such as age, sex, district of residency, educational status, previous exposure to TB, and family TB history were collected. Moreover, clinical data on the form of TB cases as either pulmonary or extrapulmonary was included. In order to assess the possible risk factors for zoonotic transmission of tuberculosis, the study participantsô knowledge, attitudes and practices towards TB both in humans and cattle, includingtheir consumption habits of raw milk/meat as well as husbandry practices were asked. Moreover, all herd owners of tuberculin tested cattle were interviewed for possible risk factors of tuberculin positivity. Data on age and sex of individual animal, herd size, origin, breed type, recent introduction of new animals into the herd, and keeping of different livestock together were collected.

2.2.2. Smear microscopy

After informed consent was obtained, two sputum samples from suspected PTB patien -ts and fine needle aspirates (FNA) samples from suspected EPTB patients were colle cted by trained laboratory technicians and pathologists, respectively.

The sputum samples were collected by standard sterile tubes. Thereafter, the samples were first digested and concentrated/decontaminated by the N-acetyl-L-cysteine-Sodium hydroxide (NALC)-NaOH) method. Smears were stained by the Ziehl-Neelsen (ZN) method and examined under oil immersion using a binocular light microscope. All smear positve samples were stored at +4°C at the study site temporarily and then transported to the Regional Health Research Laboratory Center (Bahirdar) using cold chain and then kept at +4 °C until culture was performed.

Similarly, FNA specimens were collected by pathologists and stored in cryo-tubes in phosphate buffer saline (PBS) with pH 7.2. ZN staining was performed. AFB-positive specimens were stored at -20 °C until culture was performed.

2.2.3. Mycobacterial culture

The samples were processed for culture according to the standard methods described earlier (FMoH, 2008, WH0, 1998). Both sputum and FNA samples were cultured at the Bahir Dar Regional Health Research Laboratory Centre. Briefly, sputum or FNA samples were homogenized and decontaminated with an equal volume of 4% NaOH by centrifugation at 3000 rpm for 15 minutes at room temperature. The supernatant was decanted while the sediment was neutralized with 2N HCl using phenol red as an indicator. Neutralization was achieved when the color of the solution changed from purple to yellow. About 100 µl of the suspension was inoculated on four sterile Lowenstein Jensen (LJ) medium slopes (two were supplemented with pyruvate and the other two with glycerol) and then incubated at 37^{o}C with weekly examination for growth. Cultures were considered to be negative when no visible growth was detected after eight weeks of incubation. The presence of acid-fast bacilli (AFB) in positive cultures was detected by microscopic examination using the ZN staining method. AFB positive cultures were prepared as 20% glycerol stocks and stored at ï 80°C as reference. Heat-killed cells of each AFB isolate were prepared by mixing ~2 loopfuls of cells (Ó20εl cell pellet) in 200εl distilled H_2O followed by incubation at 80°C for 45 minutes for the release of DNA after the breaking the cell wall. The killed cells were transported to the laboratory at Aklilu Lemma Institute of Pathobiology, Addis Ababa University, for molecular typing.

2.2.4. Molecular genotyping

Region of Difference (RD) 9 deletion typing

RD9-based PCR was performed according to protocols previously described (Berg *et al.*, 2009). The primers used were RD9intR, 5ô-CTG GAC CTC GAT GAC CAC TC-3ô; RD9flankR, 5ô-GCC CAA CAG CTC GAC ATC-3ô and RD9flankF, 5ô-GTG TAG GTC AGC CCC ATC C-3ô. Amplification was done by standard thermo cycler (VWR Thermo cycler, UK). The PCR amplification mixture used consisted of 10 εl HotStar Taq Master Mix (Qiagen, United Kingdom), 7.1 εl distilled water, 0.3 εl of each three primers and 2 εl of DNA template (heat killed cells), giving a total volume of 20 εl. The PCR reaction was heated at 95^{o}C for 15 min after which it was subjected to 35 cycles consisting of 95 ^{o}C for one min, 55 ^{o}C for one min, and 72 ^{o}C for one min. Thereafter, the reaction mixture was maintained at 72 ^{o}C for 10 min following which the product was removed from the thermo cycler and run on agarose gel electrophoresis for identification. For gel electrophoresis, 8 εl PCR products were mixed with 2 εl loading dye, loaded onto 1.5% agarose gel and electophoresed at 100

V and 500 mA for 45 min. The gel was then visualized using a computerized Multi-Image Light Cabinet (VWR). *M. tuberculosis* H37Rv, *M. bovis* bacille Calmette-Guérin and water were included as positive and negative controls.Interpretation of the result was based on bands of different sizes i.e. when a band size of 396 base pairs (bp) was observed the isolate was considered to be *M. tuberculosis* while when a band size of 375bp was observed the isolate was considered to be *M. bovis* (Parsons *et al*., 2002).

Spoligotyping

Isolates genetically identified as *M. tuberculosis* were spoligotyped for further strain identification. Spoligotyping makes use of the variability of the MTBC chromosomal direct repeat (DR) locus for strain differentiation as previously described (Kamerbeek*et al*., 1997). Briefly, the DR region of the isolate was amplified by PCR usingoligonucleotide primers (DRa: 5ô GGT TTT GGG TCT GAC GAC 3ô and DRb: 5ô CCG AGA GGG GAC GGA AAC 3ô) derived from theDR sequence. The DRa is biotinylated at the 5ô-end. A total volume of 25 εl reaction mixture (12.5εl Hot taq polymerase, 3.5 εl dH_2O, 2.0 εl each of primers DRa and DRb, 5 εl of DNA) was used for the PCR. The mixture was heated for 3 min and subjected to 20 cycles of 1 min at 96^{o}C, 1 min at 55^{o}C, and 30 s at 72^{o}C. The amplified product was hybridized to a set of 43 immobilized oligonucleotides, each corresponding to one of the unique spacer DNA sequences within the DR locus. The oligonucleotides were covalently bonded to a membrane (Animal and Plant Health Agency, Great Britain). For hybridization, 20 εl of the amplified PCR product was diluted in 150 εl of 2xSaline-Sodium Phosphate-EDTA (SSPE) buffer-0.1% Sodium Dodecyl Sulfate (SDS) and heat denatured. The diluted samples were pipetted in to the parallel channels in such a way that the channels of the miniblotter apparatus were perpendicular to the rows of previously deposited oligonucleotids. Hybridization was done for 60 min at 60^{o}C. After hybridization, the membrane was washed twice in 250 ml of 2x SSPE-0.5% SDS for 10 min each time at 60^{o}C and then incubated in 1:4,000 diluted streptavidine-peroxide conjugate for 45 to 60 min at 42^{o}C. The membrane was washed twice, for 10 min each time, in 250 ml of 2x SSPE-0.5SDS at 42^{o}C and rinsed with 250 ml of 2x SSPE for 5 min at room temperature. Known strains of *M. bovis* and *M. tuberculosis* H37Rv were used as positive controls, whereas Qiagen water (Qiagen Company, Germany) was used as a negative control. Hybridized DNA was detected by the enhanced chemiluminescence method. The presence or absence of spacer was used as key for the interpretation the result.

Identification of *M. tuberculosis* strains and lineages

A web-based spoligotype database which is available at http://www.pasteur guadelou pe.fr:8081/SITVITDemo/was utilized to assign shared international types (SITs) for the isolates (Brudey *et al*., 2006).The results of spoligotyping were converted into octal and binary formats. These binary and octal formats of the strains were entered into query box so that the name of the strains identified with the SIT numbers are retrieved from the database if the spoligotype pattern of the strain in question fits the pattern that has already been registered in the database. If the pattern of the strain in question has not been registered previously, no name was retrieved, and hence the stra _in was considered as an óorphanô. Two or more mycobacteria isolates sharing identic -al spoligotype patterns in the study were identified as clusters, whereas single spoligo patterns were considered as unique strains. An online tool Run TB Lineage http://tbin sight.cs.rpi.edu/run_tb_lineage.html was also used to predict the lineages/families.

2.2.5. Drug sensitivity test (DST) by molecular method:GenoType *MTBDRplus* assay

It is a molecular genetic assay for identification of *M. tuberculosis* complex and its resistance to Rifampicin (RIF) and Isoniazide (INH) from clinical specmens and cultivated samples. The assay is based on the DNA-STRIP technology. The whole procedure involves a multiplex PCR amplificaton with biotinylated primers, and a reverse hybridization. This assay detects for the abscence and/or presence of wild type (WT) and/or mutant (MUT) DNA sequences with in specific region of three genes: the rpoB gene (coding for the ɓ-subunit of the RNA polymeraze), for the identification of RIF resistance; the katG gene (coding for the catalase peroxidase), for high level INH resistance; and the promoter region of the inhA gene (coding for the NADH enoylACP reductase), for low-level INH resistance. Each strip contains 27 reaction zones. The procedure of the test was performed based on the manufacturerôs instructions (Hain Life sciences, Nehren, Germany).

PCR based amplification of genomic DNA: For a single PCR reaction, 10 µl amplification mix A containing 10ml buffer, nucleotides, and DNA polymerase was mixed with 35 µl of amplification mix B containing $MgCl_2$, the biotinylated primers,and dye. Then, 5 µl of *M. tuberculosis* DNA was addedto the mixture, making the final volume of PCR mix to be50 µl. The PCR consisted of 15 min of denaturing at 95 ^{0}C, followedby 10 cycles of 30s at 95 ^{0}C and 120s at 58 ^{0}C, followed by 20 additional cycles of 25s at 95 ^{0}C, 40s at 53 ^{0}C, and 40sat 70 ^{0}C, with a final extension at 70 ^{0}C for 8min.

Hybridization: For hybridization, 20 µl of the amplification products weremixed with 20 µlof the denaturing reagent (provided with the kit) and denaturing was performed for 5min in each of the plasticwell.Thereafter, 1 ml of prewarmed hybridization buffer was added into each well and one strip was placed in each well.The hybridization was performed at 45^0C for 30 min, followedby twowashing steps. For colorimetric detection of hybridized amplicons, streptavidin conjugated with alkaline phosphatase was added after which a substrate buffer was added. After final washing, strips were air dried and fixed on paper. The DNA of the standard strain H37Rv and molecular-grade water were used as positive and negative controls, respectively.

Detection of drug resistance conferring gene mutations and interpretation of results

Each strip consists of 27 reaction zones (bands) with six controls including conjugate control (CC), amplification control (AC), *M. tuberculosis* complex, rpoB locus control, katG locus control, and inhA locus control. The remaining 21 reaction zones are WT and mutation reaction zones including eightrpoB WT and four MUT probes, one katG WT and two MUTprobes, and two inhA WT and four MUT probes. Results were interpreted according to the manufacturerôs instructions (Hain Life sciences, Nehren, Germany). In brief, the presence of CC bands indicates the efficiency ofthe conjugate and substrate, the presence of AC bands indicates the efficiency of DNA extraction and PCR procedures, and the presence of the *M. tuberculosis* complex band indicates that the tested bacterium belongs to the *M. tuberculosis* complex. The three respective locus control bands (rpoB, katG, and inhA) indicate the presence of the specific gene region.The absence of the WT band is usually accompanied by the presence of MUT, which indicates resistance and the presence of all WT bands without the MUT band indicates susceptible isolate. In rare cases, lack of WT band(s) without a corresponding MUT band could be observed due to uncommon mutations in the probe region and the presence of both WTand MUT bands in the same stripe might be an indication for the presence of heteroresistance or mixed infection.

2.2.6. Single intradermal comparative tuberculin test (SICCTT)

Tuberculin skin tests were performed for detectng tuberculosis on cattle owned or had close association with confirmed TB patients. The SICCTT was done using both bovine and avian mycobacterium purified protein derivative (PPD) (obtained from Prionics Lelystad B. V., The Netherlands). Two injection sites were chosen in the middle of the side of the neck, one above the other, separated by about 12 cm. approximately, 2cm radius hairs were shaved around the injection sites. The thickness of the skin fold at both injection sites were measured and recorded using a caliper before and after 72 hours of PPD injection. An aliquot of tuberculin containing 2500IU/0.1ml bovine PPD was injected intradermally in the lower injection site; similarly, 2500IU/0.1ml avian PPD was injected in the upper site. Bovine positive reactors were determined using the formula $[(Bov_{72}-Bov_0)-(Av_{72}-Av_0)]$. Bov_0 and Av_0 indicated skin thickness before injection of bovine and avian tuberculin, respectively, and Bov_{72} andAv_{72} were the corresponding skin fold thickness measurements after 72 hours of the injections. A cattle was considered to be positive for BTB if the skin reaction at the bovine-PPD injection site minus the skin reaction at the avian-PPD site is$\geq$ 4mm (OIE, 2009) or$\geq$2mm cut off (Ameni *et al*., 2008).

2.3. Quality Control of Laboratory Procedures

M. tuberculosis H37Rv (ATCC 27294) and *M. bovis* (AF 61/2122/97) were included for quality control in DST, RD9 deletion typing, and spoligotyping. Laboratory procedures were done following standard operational procedures. The activities performed to undertake the present study are summarized by the flow chart below (Figure 10).

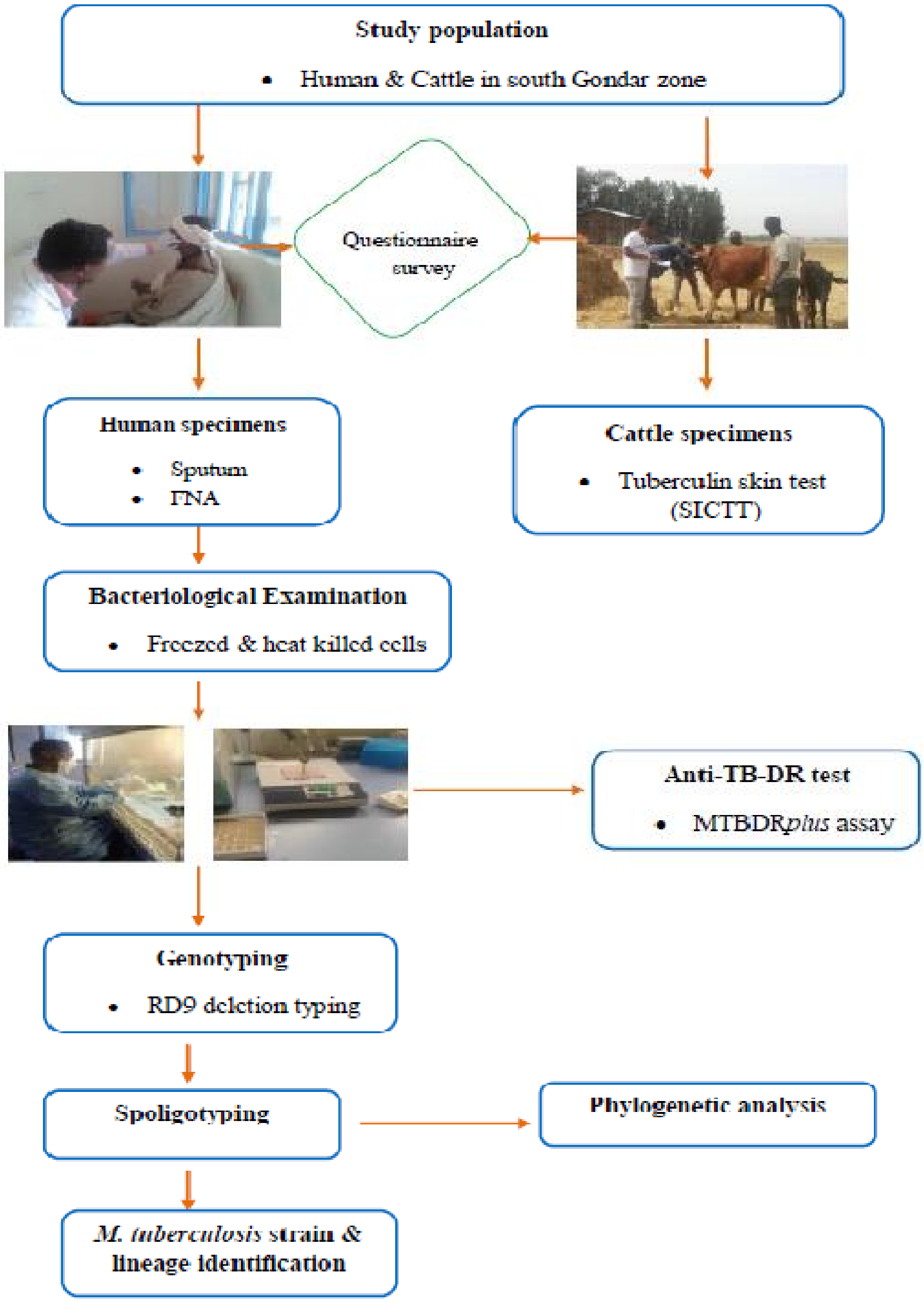


Figure 10. Flow chart demonstrating the activities performed to undertake the study.

2.4. Statistical Data Analysis

Data were entered in to Excel file format andtransferred in to SPSS software version 25 for statistical analysis. Descriptive statistics were used to depict the demographic variables. Chi-square (χ^2) tests were used to test differences in proportions and further used to determine association between categorical variables with TB prevalence, drug sensitivity paterns and genotypes of *M. tuberculosis* isolates. Bivariate and multivariate logistic regressions were used to determine the association between background variables with awareness to zoonotic TB, tuberculin reactivity in cattle, genotype clustering and drug sensitivity patterns of mycobacteria isolates. Results were considered statistically significant whenever p-value was less than 5%.

2.5. Ethical consideration

Ethical clearance for the study was obtained from the Ethical Committee of Addis Ababa University, College of Natural and Computational Sciences (Ref.CNSDO/491/ 07/15). In addition, written permission was sought from the Amhara Regional Health Bureau Research Ethical Committee (Ref. HRTT/1/271/07).All human subjects consented with a written form (which was translated into the local language (óAmharicö) and agreed to participate in the study. In case of participants under the age of 18 years, consent was obtained from their parents/guardians (Appendix I).

3. RESULTS

3.1. Smear positive human TB in individuals visiting health facilities in the study area

3.1.1. Sociodemographic characteristics of the study participants

A total of 3168 TB-suspected individuals, with a response rate of 93.2% (2,953/3168) participated in the study. Of these, 1,733 (58.7%) were males and 1, 220 (41.3%) were females. Other sociodemographic characteristics such as patient origin, age, education status, status of prior exposure to TB within the family members and their milk consumption habits are presented and thier association with TB prevalence has been determined (Table 2).

Table 2. Socio-demographic characteristics of study participants and their association with tuberculosis prevalence, South Gondar Zone, northwest Ethiopia (N= 2,953) (2015-2017).

Characteristics	Number, n (%)	AFB smear result Positive (%)	Negative (%)	χ^2(df)	p-value
Patient origin (Districts)					
Dera	485(16.4)	48 (9.9)	437 (90.1)	62. 80 (7)	<0.001
Ebinat	256(8.7)	17(6.6)	239(93.4)		
Este	308(10.4)	10 (3.2)	298 (96.8)		
Farta	424(14.4)	8 (1.9)	416 (98.1)		
Fogera	494(16.7)	56 (11.3)	438 (88.7)		
Gayint	367(12.4)	9 (2.5)	358 (91.0)		
Libo kemkem	375(12.7)	28 (7.5)	347 (92.5)		
Simada	244(8.3)	10 (4.1)	234 (95.9)		
Age group (year)					
<18	428(14.5)	21 (4.9)	407 (95.1)	35.46 (4)	<0.001
18-30	676(22.9)	66 (9.8)	610 (90.2)		
31-43	757(25.6)	50 (6.6)	707(93.4)		
44-56	486(16.5)	37 (7.6)	449 (92.4)		
>56	606(20.5)	12 (2.0)	594 (98.0)		
Sex					
Male	1,733(58.7)	100 (5.8)	1,633 (94.2)	1.98(1)	0.159
Female	1,220(41.3)	86 (7.0)	1,134 (93.0)		
Education status					
Illiterate	1654(56.0)	117 (7.1)	1,537 (92.9)	8.06 (4)	0.089
Adult education	225(7.6)	11 (4.9)	214 (95.1)		
Primary school	790(26.8)	37 (4.7)	753 (95.3)		
Secondary school	169(5.7)	15 (8.9)	154 (91.1)		
Higher education	115(3.9)	6 (5.2)	109 (94.8)		
TB history in the family					
Yes	2,187 (74.1)	134 (6.1)	714 (93.9)	182.03(1)	0.84
No	766 (25.9)	52 (6.8)	2,053 (93.2)		

3.1.2. Mycobacterium culture yield

Out of the total 186 smear positive mycobacterial isolates obtained from patients clinically diagnosed at health facilities in the study area, 111 (59.7%) were culture positive. Sputum samples were collected at woreda health centers, rural health posts and referal hospitals. However, all the FNA samples were collected from extrapulmon _ary TB suspects who were refered to zonal or regional referal hospitals, where the pathological examination service were available. The Sputum and FNA samples taken from 70 and 116 cases were cultured with a culturing yield of 64.3% (45/70) and 56.9% (66/116) AFB positive isolates, respectively.

3.1.3. Prevalence of smear positive TB among the study participants

The overall prevalence of smear positive of all forms of TB was 6.3% (186/2953). The study showed variability in TB prevalence among the different socio-demographic characteristics such as sex, age and patient origin (districts). Accordingly, males (3.4% (100/2953), age groups from 18 to 30 years (2.2% (66/2953), and Fogera district (1.8% (56/2953), had the highest prevalences. Moreover, the majority of smear positive cases (72.0%) had history of TB in their family (Table 2). EPTB was clinically characterized in about 62.4% (116/186) TB-positive cases in the study area, and 86.0% (160/186) of them were newly diagnosed for TB (Table 3).

Table 3. Number of smear positive TB cases categorized by sex, patient category and TB type for study participants, South Gondar Zone, northwest Ethiopia (2015-2017).

	Patient category		**TB type**	
Sex	New, n (%)	Retreatment, n (%)	PTB, n (%)	EPTB, n (%)
Male	83 (44.6)	17 (9.1)	37 (19.9)	63 (33.9)
Female	77(41.4)	9 (4.9)	33 (17.7)	53 (28.5)
Total	**160 (86.0)**	**26 (14.0)**	**70 (37.6)**	**116 (62.4)**

3.1.4. Risk factor assessment

Patients orign and age groups were significantly associated (p<0.001) with TB prevalence. However, sex, educational status, TB history in the family and raw milk consumption habits were not significantly associated with the occurrence of all forms TB (p> 0.05 for all) (Table 2). Similarly, no significant association was observed between any of the different demographic or clinical characteristics and the prevalence of EPTB among the study participants (p>0.05 for all) (Table 4).

Table 4.Association of risk factors to EPTB in humans, South Gondar Zone, northwest Ethiopia (2015-2017).

Characteristics	All forms TB* (%)	EPTB (%)	p-value
Age group (year)			
<18	21(11.3)	17(81.0)	
18-30	66 (35.4)	43(65.2)	0.56
31-43	50 (26.9)	25(50.0)	0.23
44-56	37 (19.9)	25(67.6)	0.66
>56	12 (6.5)	6(50.0)	0.41
Sex			
Male	100 (53.8)	63(63.0)	
Female	86 (46.2)	53(61.6)	1.00
Education status			
Illiterate	117 (62.9)	71(60.7)	
Adult education	11(5.9)	8(72.7)	0.71
Primary school	37(19.9)	24(64.9)	0. 82
Secondary school	15(8.1)	9(60.0)	1.00
Higher education	6 (3.2)	4(66.7)	0. 88
TB history in the family			
Yes	52 (28.0)	37(71.2)	
No	134 (72.0)	79(59.0)	0.46
Consumption of raw milk			
Yes	133 (71.5)	87(65.4)	
No	53 (28.5)	29(54.7)	0.50
Patient category			
New	160 (86.0)	103(64.4)	
Retreatment	26 (14.0)	13(50.0)	0.48

*Both pulmonary and extrapulmonary TB

3.2. Molecular typing of *M. tuberculosis* isolates

3.2.1. RD9 deletion typing patterns of *M. tuberculosis* strains

After smear microscopy and mycobacterial culture, a total of 106 culture-positive mycobacterialisolates were tested by RD9-based PCR. The test revealed that all isolates had intact RD9 locus and were subsequently classified as *M. tuberculosis* (Figure 11). No other species of MTBC was detected.

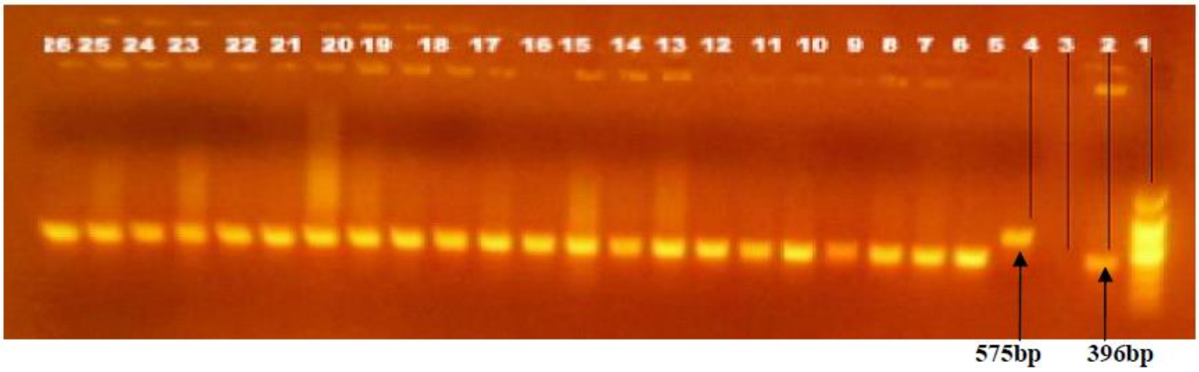


Figure 11. Electrophoretic separation of PCR products by RD9 deletion typing of 22 mycobacteria isolates from sputum and FNA human samples: Lane 1 DNA ladder, 2 *M. tuberculosis* control (396 base pair), 3 molecular grade water (negative control), 4 *M. bovis* control (575 bp), lane 5-26 are culture isolates of *M. tuberculosis* from human tuberculosis patients designated with their sample code as: 5FE1, 6FE2,7FE3,8FE4, 9FE7, 10 FE11, 11FE12,12FE15, 13FE16,14FE19,15FE21, 16FE23, 17FE24, 18FE25, 19FE27, 20FE28, 21FE31, 22FE33, 23FE36, 24FE38, 25FE47, 26FE48.

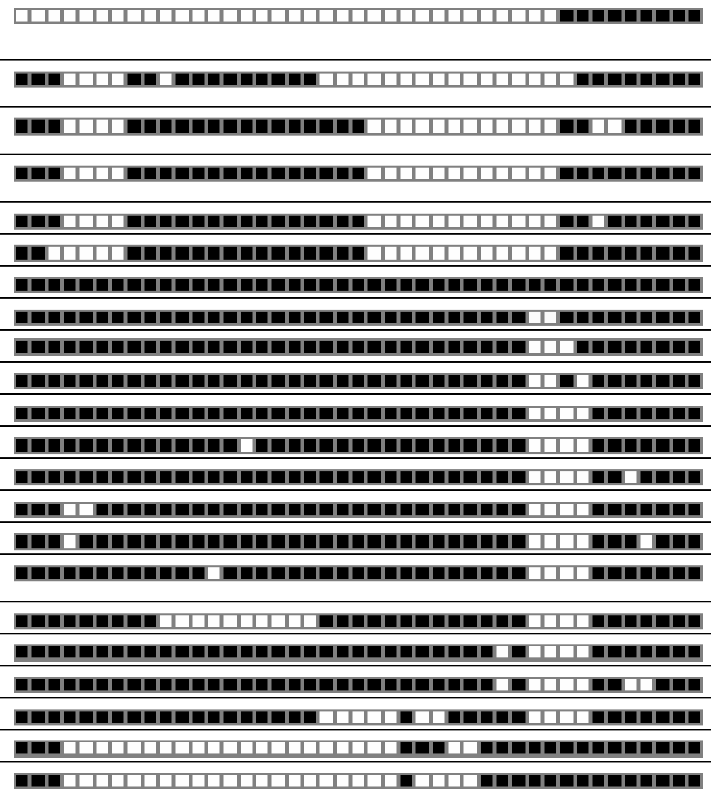
3.2.2. Spoligotyping patterns of *M. tuberculosis* isolates

The patterns of 96 isolates were interpretable and grouped into 35 different spoligotype patterns. From the 35 patterns, 22 patterns were shared types and consisted of 79 isolates (Table 5). The dominantly identified SITs were SIT53 (Lineage 4), SIT149 (Lineage 4), and SIT428 (Lineage 3), each consisting of 18, 14 and 12 isolates, respectively (Table 5). These three SITs consisted of 45.8% of the total isolates. The remaining 13 were not registered in the database and were óorphansô strains (Table 6).

The two dominant major lineages identified in this study were Lineage 4 (Euro-American, 62.5%) and Lineage 3 (Central Asia, 26.0%). MTBC strains identified as lineage 7 (Afri), lineage 1(East African Indian) and lineage 2 (Beijing) were only identified in seven, three and one patients, respectively. The three dominant -ly identified families were T, CAS and Manu, each consisting of 46.9%, 24.0% and 10.4% of the isolates, respectively (Tables 5 and 6).

Table 5.Spoligotype patterns of 22 shared types and their corresponding lineages/families identified from a total of 96 *M. tuberculosis* strains isolated in South Gondar Zone, northwest Ethiopia (2015-2017).

SITs	**No. of Isolates**	**Spoligotype families**	**Lineages**	**Octal number**	**Spoligotype pattern**
1	1	Beijing	Lineage 2 (EAS)	000000000003771	
21	1	CAS1_Kili	Lineage 3 (CAS)	703377400001771	
25	1	CAS1_Delhi		703777740003171	
26	4	CAS1_Delhi		703777740003771	
428	12	CAS1_Delhi		703777740003371	
952	1	CAS1_Delhi		603777740003771	
523	1	Manu1	Lineage 1(EAI)	777777777777771	
54	1	Manu2	Lineage 4 (EA)	777777777763771	
1196	1	Manu2		777777777761771	
1690	1	Manu2		777777777762771	
53	18	T		777777777760771	
118	1	T		777767777760771	
245	1	T		777777777760671	
358	4	T		717777777760771	
848	1	T2		737777777760731	
37	1	T3		777737777760771	
149	14	T3-ETH		777000377760771	
50	2	H3		777777777720771	
134	3	H3		777777777720631	
41	3	Turkey		777777404760771	
910	2	AFRI	Lineage 7 (West African I)	700000007177771	
1729	5	AFRI		700000004177771	



SIT; Shared International Type, OR; Orphan,. CAS: Central Asian Strain; H: Haarlem;AFRI; Africa, , EA; Euro-American, EAS; East-Asian, EAI; East African-Indian. The colors represented the different families of *M. tuberculosis* isolates. Filled boxes represent positive hybridization while empty boxes represent absence of spacers.

Table 6:Spoligotype patterns of 13 orphan strains and their corresponding lineages/families identified from a total of 96 *M. tuberculosis* isolates collected in tuberculosis patients in South Gondar Zone, northwest Ethiopia (2015-2017).

SITs	**No. of Isolates**	**Spoligotype families**	**Lineages**	**Octal number**	**Spoligotype pattern**
OR1	1	CAS1_Delhi	Lineage 3 (CAS)	703767740007771	
OR2	2	CAS1_Delhi		703777700001171	
OR3	1	CAS1_Delhi		703677740003171	
OR4	1	T	Lineage 4 (EA)	777367777760771	
OR5	1	T		007777777760771	
OR6	1	T		776601777760771	
OR7	1	T3		776737777760771	
OR8	1	X1		777376777760771	
OR9	1	H3		777763703420771	
OR10	3	Manue2		717777777763771	
OR11	1	Manu ancestor	Lineage 1 (EAI)	703367747177771	
OR12	2	Manu1		717777777777771	
OR13	1	H		700002004140371	

3.2.3. Spatial distribution of *M. tuberculosis* lineages in South Gondar Zone

Among the major lineages of *M. tuberculosis,* Lineage 3 and Lineage 4 were found to be abundantly distributed in all districts of the zone (except the absence of lineage 3 in Este district). CASI-Delhi (which consistes the study area-specific SIT428 strain) and T3-ETH families were the major constituents of Lineage 3 and Lineage 4, respectively. Lineage 7 was isolated in TB patients in Dera (2 isolates), Este (1 isolate), Farta (1 isolate), Gayint (1 isolate) and Simada (1 isolate) districts (Figure 12).

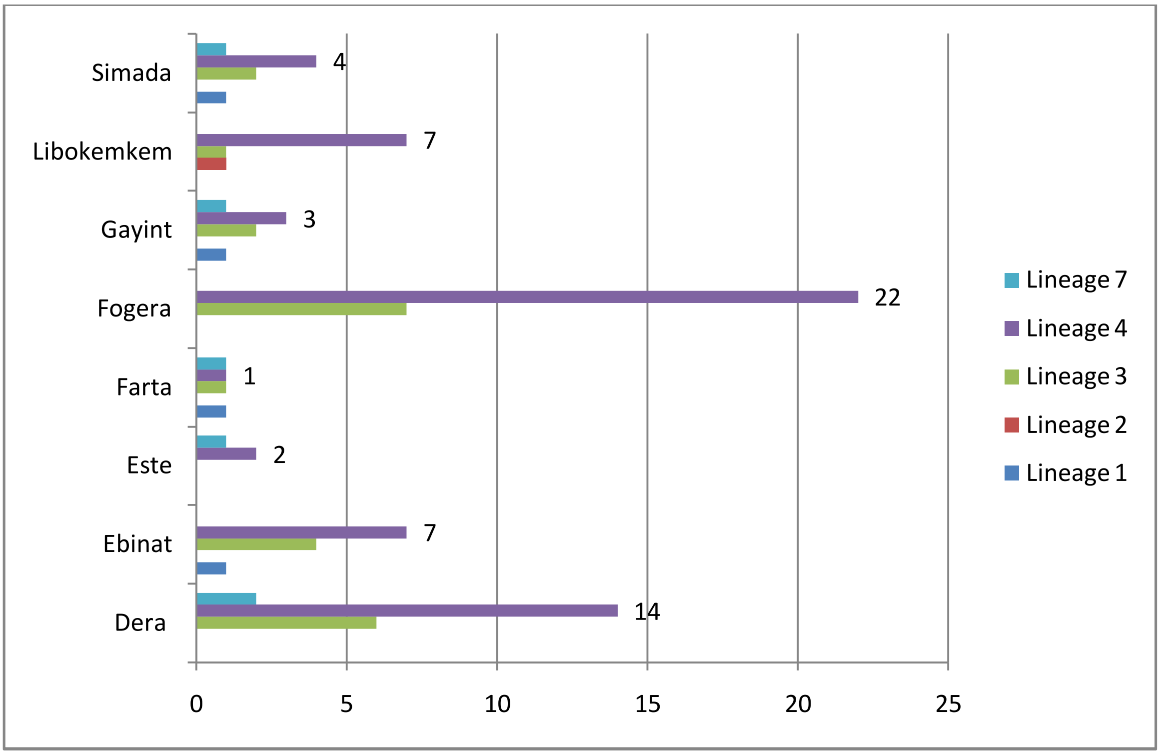


Figure 12. Distribution of *M. tuberculosis* lineages in the districts of South Gondar Zone (2015-2017).

3.2.4. Association of patient characteristics with *M. tuberculosis* genotype clustering

Higher percentages of the total 96 *M. tuberculosis* isolates were identified from Dera District(30.2%) followed by Fogera District (22.9%). The clustering rates of strains isolated from these two districts were 89.7 and 72.7, respectively. The least number of *M.*

tuberculosis isolates were identified from Este (3.1%) with a clustering rate of 33.3% (Table 7).

Patient origin and educational background were observed to be significantly associated with patient characteristics and clustering level of the strains. TB patients from Este and Ebinat districts were less likely to have clustered strains than those from Dera[(Este vs. Dera: odds ratio [AOR] =0.32; 95% CI:0.00-2.07**;** p =0.01)] and [(Ebinat vs. Dera: AOR= 0.12; 95% CI: 0.01-0.69;p =0.01)]. Similarly, as compared to Dera, there was lower odd of strain clustering in samples from Gayint district (Gayint vs. Dera: AOR= 0.16; 95 % CI:0.01-1.09;p =0.03). Clustering of strains was more than three times higher in sample from illiterates than from those with secondary school education (Secondary vs. Illiterates: AOR=0.27; 95% CI:0.04-1.16;p = 0.05). Clustering level, however, was not significantly associated with the other demographic and clinical characteristics of the study participants (Table 7).

A total of 74 (77.1%) isolates were grouped into 13 different clusters of strains which ranged from 2 to 18 isolates. The remaining 22 (22.1%) isolates had single strains (unique strains). The overall clustering rate was 77.1 (Table 7). Higher number (26) of clustered strains occurred in the strains of the Dera district (Table 7).

Table7. Demographic and clinical characteristics of the study participants and their association with spoligotype clustering of TB isolates from South Gondar Zone, northwest Ethiopia (2015-2017).

Demographic characteristics	Number of isolates (%)	Number of isolates clustered Vs unique			COR (95% CI)	AOR (95%CI)	p- value
		Clustered	Unique	Clustering rate			
Patient origin(District)							
Dera	29 (30.2)	26	3	89.7	1.00		
Fogera	22 (22.9)	16	6	72.7	0.30(0.06-1.40)	0.35(0.01-1.93)	0.11
Libo-kemkem	10 (10.4)	7	3	70.0	0.26(0.04-1.63)	0.14 (0.02-1.65)	0.13
Simada	9 (9.4)	7	2	77.8	0.40(0.05-2.90)	0.45(0.01-3.71)	0.35
Ebinat	10 (10.4)	5	5	50.0	0.11(0.02-0.64)	0.12(0.01-0.69)	0.01
Farta	6 (6.3)	4	2	66.7	0.23(0.02-1.84)	0.28(0.01-2.37)	0.14
Gayint	7 (7.3)	4	3	57.1	0.15(0.02-1.04)	0.16(0.01-1.09)	0.03
Este	3 (3.1)	1	2	33.3	0.05(0.00-0.84)	0.32(0.00-2.07)	0.01
Sex							
Male	50 (52.1)	33	17	66.0	0.47(0.18-1.20)	0.52(0.13-1.73)	0.11
Female	46 (47.9)	37	9	80.4	1.00		
Age (years)							
5-17	9 (9.4)	7	2	77.8	1.00		
18-30	37 (38.5)	27	10	73.0	0.77(0.13-4.35)	0.96(0.20-4.80)	0.76
31-43	28 (29.1)	19	9	67.9	0.60(0.10-3.50)	0.20(0.01-4.10)	0.57
44-56	16 (16.7)	13	3	81.3	1.23(0.16-9.25)	1.05(0.05-9.27)	0.83
>56	6 (6.3)	4	2	66.7	0.57(0.05-5.77)	0.40(0.02-5.84)	0.63
Education status							
Illiterate	64 (66.7)	48	16	75.0	1.00		
Adult education	4 (4.1)	2	2	50.0	0.33(0.04-2.56)	0.06(0.01-2.20)	0.27
Primary school	16 (16.7)	14	2	87.5	2.33(0.47-11.39)	2.06(0.29-11.10)	0.28
Secondary level	9 (9.4)	4	5	44.4	0.26(0.06-1.11)	0.27(0.04-1.16)	0.05
College level	3 (3.1)	2	1	66.7	0.66(0.05-7.85)	0.75(0.12-8.28)	0.74
Patient category							
New	81 (84.4)	58	23	71.6	0.63(0.16-2.44)	0.63(0.13-2.63	0.50
Retreatment	15 (15.6)	12	3	80.0	1.00		
Family TB history	33 (34.4)	26	7	78.8	1.00		
Yes	63 (65.6)	44	19	69.8	0.62(0.23-1.68)	0.35(0.05-1.30)	0.34
No							
Clinical presentation							
PTB	34 (35.4)	26	8	76.5	1.00		
EPTB	62 (64.6)	44	18	71.0	0.75(0.28-1.97)	0.58(0.13-1.95)	0.56
Total	**96 (100)**	**74**	**22**	**77.1**			

3.3. Molecular detection of *Mycobacterium tuberculosis* sensitivity to Rifampicin and Isoniazid

3.3.1. Drug resistance patterns among the different patient characteristics

The drug sensitivity test was performed for 111 culture positive *M. tuberculosis* isolates obtained from all forms of TB patients (both pulmonary and extrapulmonary TB patients). Of which, 20 (18.0%) were resistant to atleast one of the two drugs, RIF and/or INH. Younger adults aged between 18-30 years constituted the greatest number of resistant cases (9/20). (Table 8).

Table 8.Socio-demographic characteristics of study participants and their association with drug sensitivity patterns of mycobacterial isolates,South Gondar Zone, Amhara Region, northwest Ethiopia (2015-2017).

	Any drug resistance			**COR (95% CI)**	**p-value**
Characteristics	**Yes, n (%)**	**No, n (%)**	**Total n (%)**		
Sex					
Male	10 (17.5)	47 (82.5)	57 (100)	1.00	
Female	10 (18.5)	44 (81.5)	54 (100)	1.06 (0.40-2.81)	1.00
Age					
<18	2 (18.2)	9 (81.8)	11(100)	1.00	
18-30	9 (21.4)	33 (78.6)	42 (100)	1.22 (0.22-6.72)	0.813
31-43	3 (8.8)	31(91.2)	34 (100)	0.43 (0.06-3.02)	0.390
44-56	4 (22.2)	14 (77.8)	18 (100)	1.28 (0.19-8.53)	0.794
>56	2 (33.3)	4 (66.7)	6 (100)	2.25 (0.22-22.14)	0.481
Patient category					
New cases	15 (16.7)	75 (83.3)	90 (100)	1.00	
Retreatment	5 (23.8)	16 (76.2)	21 (100)	1.56 (0.49-4.92)	0.443
Family TB history					
Yes	6 (16.7)	30 (83.3)	36 (100)	1.00	
No	14 (18.7)	61 (81.3)	75 (100)	1.14 (0.40-3.28)	0.797
Clinical presentation					
PTB					
EPTB	11(25.0)	33 (75.0)	44 (100)	1.00	
	9 (13.4)	58 (86.6)	67 (100)	0.46 (0.17-1.23)	0.120

COR; crude odds ratio, CI; confidence interval

Of 90 newly diagnosed TB cases, 75 (83.3%) were susceptiable to both RIF and INH (RIFs, INHs) but the remaining 15 (16.7%) were INH mono-resistant (RIFs, INHr).

Similarly, 23.8% (5/21) of the retreatment cases were resistant to either ofRIF and INH(Table 8).

Despite the observed differences in the proportions of drug resistant cases,no statistically significant association was observed between demographic and clinical characteristics of patients with drug resistance. From a total of 21 retreatment TB cases, 76.2% (16/21) were suscebtible to both drugs and the remaining 23.8% (5/21) were resistant to atleast one of the two anti-TB drugs (Table 8).

MDR-TB cases (resistance to both RIF and INH) were observed in 1.8% (2/111) of the cases. Both cases were detected from retreatment TB patients. No RIF mono-resistant (RIFr, INHs) case was detected in any of the disease or patient categories (Table 9).

Table 9. Resistance patterns of *M. Tuberculosis*isolates to INH and RIF with TB patient category, South Gondar Zone, Amhara Region, northwest Ethiopia (2015-2017).

Drug resstance pattern	New cases (%) (n=90)	Retreatment (%) cases (n=21)	Total (%) (N = 111)
Any Susceptible	75 (83.3)	16(76.2)	91(82.0))
Any resistance	15 (16.7)	5 (23.8)	20 (18.0)
INHmono-resistance	15 (16.7)	3 (14.3)	18 (16.2)
INH Susceptible	75 (83.3)	16 (76.2)	91(82.0)
RIF mono-resistance	0 (0.0)	0 (0.0)	0 (0.0)
RIFSusceptible	90 (100)	19 (90.5)	109 (98.2)
MDR (INH+RIFresistance)	0 (0.0)	2 (9.5)	2 (1.8)
Hetroresistance	6 (6.7)	0 (0.0)	9 (8.1)

INH:Isoniazid, RIF: Rifampicin, MDR: Multidrug resistance

3.3.2. Drug resistance-confering gene mutations

From the total samples tested for drug sensitivity, 18.0% (20/111) of them showed atleast one gene mutation. Among the three targeted genes (*katG*, *rpoB* and *inhA*), mutations were observed only in the *katG* and *rpoB* genes, confering a high level of INH resistance and RIF resistance, respectively. All INH-resstance confering mutations occured only at *katG* gene (*katG* MUT1). In the present study, no *inhA* gene mutaion was observed. About 1.8% (2/111) RIF-resistance confering gene mutations occured and resulted from the absence of *rpoB* WT7, without the presence of MUT3 in both specimens. Both the RIF-resistance confering gene mutations at rpo*B* occured together with INH-resistance confering gene mutations at *katG* genes, signaling MDR-TB pattern. Among the 20 INH-resistance confering mutations, specific mutations (the WT probe was missing with the presence of the corresponding MUT1 probe) of the *katG* gene were observed in 12 isolates. In six INH-resistant isolates, unexpected mutation patterns were observed, both the WT and the corresponding MUT1 probes of the *katG* gene were positive,signaling a hetroresistance pattern and considered as órareô mutations (Table 10). WT7 probes of the *proB* gene were missed without the presence of the corresponding MUT probes and considered as óunknownô mutations (Table 10).

Table 10. Distribution of target gene mutations and resistance patterns in anti-TB drug resistant *M. tuberculosis*isolates,South Gondar Zone, Amhara Region, northwest Ethiopia (2015-2017).

		Target genes			
Isolates	*rpoB*	*katG*	*InhA*	Type of mutation	Drug resistance pattern
FE31	WT1-8	MUT 1	WT1,2	specific	RIFs INHr
FE34	WT1-8	MUT1	WT1,2	specific	RIFs INHr
FE50	WT1-8	WT, MUT 1	WT1,2	rare	RIFs INHr*
FE51	WT1-8	MUT1	WT1,2	specific	RIFs INHr
FE54	WT1-8	MUT1	WT1,2	specific	RIFs INHr
FE55	WT1 8	MUT1	WT1,2	specific	RIFs INHr
FE56	WT1-8	WT, MUT1	WT1,2	rare	RIFs INHr*
FE72	WT1-8	WT, MUT1	WT1,2	rare	RIFs INHr*
FE74	WT1-8	MUT1	WT1,2	specific	RIFs INHr
FP12	WT1-8	WT, MUT1	WT1,2	rare	RIFs INHr*
WHC02	WT1-8	MUT1	WT1,2	specific	RIFs INHr
WHC13	WT1-8	MUT1	WT1,2	specific	RIFs INHr
WHC14	WT1-8	MUT1	WT1,2	specific	RIFs INHr
WHC405	WT1-8	WT, MUT1	WT1,2	rare	RIFs INHr*
WHC322	WT1-6, 8,	MUT1	WT1,2	unknown	RIFr INHr**
WHC710	WT1-6, 8,	MUT1	WT1,2	unknown	RIFr INHr**
AZ05	WT1-8	WT, MUT1	WT1,2	rare	RIFs INHr*
AZ06	WT1-8	MUT1	WT1,2	specific	RIFs INHr
WHN02	WT1-8	MUT1	WT1,2	specific	RIFs INHr
255A	WT1-8	MUT1	WT1,2	specific	RIFs INHr

Hetroresi*stance;Multidrug resistance

WT: Wild type; MUT: Mutant type; RIF: Rifampicin; : INH: isoniazid; the subscripts ósô and órô refer to : sensitive and resistance, respectively.

3.3.3. Association of drug resistance pattern and *M. tuberculosis* lineages

The majority of resistant strains for any of anti-TB drugs (RIF, INH, RIF and INH) belonged to the EA major lineage, 16.7% (16/96), sub-lineage T,10.4% (10/96), orphan dominant strains, 6.3% (6/96) and clustered strains, 13.5% (13/96). The two MDR cases belonged to the EA major lineage and T3-ETH sub-lineage.

A statistically significant association (χ^2: 9.3; p=0.02) was observed between any anti-TB drug resistance and major lineages of *M. tuberculosis* strains. Although the statistical association between the categories of sub-lineages, dominant strains and clustering level with any drug resistance of the isolates was not significant, differences in proportion were observed (Table 11).

Table 11. Association of drug resistance with genotypeof *M. tuberculosis* isolates from TB patients, South Gondar Zone, Amhara Region, northwest Ethiopia (2015-2017).

Characteristics	**Variables**	**Any drug resistance**		**Total**	**χ^2(df)**	**p- value**
		Yes/No				
Major lineage						
EA		16	43	59	9.30 (3)	0.02
EAI		0	25	25		
EAS		0	1	1		
IO		0	2	2		
Sublineages/families						
AFRI		0	7	71	4.07(8)	0.08
Beijing		0	1	1		
CAS		0	23	23		
H		1	6	7		
Manu		3	7	10		
T		10	20	30		
T3-ETH		4	10	14		
Turkey		0	3	3		
X		0	1	1		
Dominant strains						
Orphan		6	14	20	4.30 (3)	0.23`
SIT53		4	14	18		
SIT149		3	9	12		
SIT428		0	12	12		
Clustering						
No		5	20	25	0.034 (1)	0.852
Yes		13	58	71		

EA: Euro-American; EAI: East-African-Indian; IO: Indo-Oceanic; EAS: East-Asian; CAS: Central Asian Strain; H: Harlem; AFRI: African; SIT: spoligotype international types.χ^2: Chi-square, df: degree of freedom.

3.4. Prevalence of TB in cattle and its zoonotic transmission to small holder farmers in the study area

3.4.1. Characteristics of the study cattle

Majority of the cattle were females, accounting for54.2% (258/476) of the population screened. Cattle within the age range of 5-10 years had the greatest share (47%) from the total cattle tested with a mean age of 5.5 years. Many of the cattle were Zebubreed while only 6.1% (29/476) were cross breed (Table 12).

3.4.2. Prevalence of TB in cattle

Bovine TB prevalence in the animalswas 1.6% (5/315) and 1.2% (2/161) at Ó2mm cut-off value in TB positive and TB free households, respectively. Using the same cut-off value, 7.9% (5/63) and 6.3% (2/32) herd prevalence was recorded in cattle owned by TB positive and TB free households, respectively. The overall animal and herd prevalence were 1.5% (7/476 and 7.4% (7/95), respectively. However, non of the tested cattle were positive for bovine TB at the international cut-off value of >4mm.

3.4.3. Risk factors for bovine TB

Risk factor analysis to the occurrence of bovine TB in cattle revealed that age groups between 5-10 years were more reactive to tuberculin test than younger age groups (AOR=3.1; 95% CI: 0.35-35.69; p=0.16). Cattle with apparently good (AOR=8.53; 95% CI: 0.85-83.34**;** p =0.02**)** and medium (AOR= 3.00; 95% CI: 0.27-28.38; p = 0.33) body conditions were more likely to be reactive to the tuberculin test as compared to those with apparently poor body condition, and the difference was statistically significant (p<0.05). Although the difference was not statistically significant (p>0.05, the odd of bovine TB that cattle owned by TB positive cases was slightly higher than those owned by TB free households (AOR=1.39; 95% CI: 0.31-

7.10; p = 0.76). Despite the observed differences, sex, breed type, source of cattle and households TB status were not significantly associated with the occurrence of BTB in the present study (Table 12).

Table 12.Association of host risk factors with bovine tuberculin test reactivity in cattle based on a >2mm cutoff value, South Gondar Zone, northwest Ethiopia (2015-2017).

Characteristics	**Tuberculin test (n=476)**		**Total (%)**	**COR (95% CI)**	**AOR (95% CI)**	**p-value**
	Positive	Negative				
Age of cattle (years)						
<5	1	160	161(33.8)	1		
5-10	5	203	208 (43.7)	4.11 (0.47- 35.55)	3.10 (0.35-35.69)	0.16
>10	1	106	107(22.5)	1.58 (0.09-25.45)	1.69 (0.16-25.93)	0.74
Sex						
Male	2	215	217 (45.6)	1		
Female	5	254	259 (54.4)	2.11(0.40-11.01)	2.16 (0.35-12.21)	0.36
Breed type						
Local	6	441	447 (94.0)	1		
Cross	1	28	29 (6.0)	2.62 (0.30-22.56)	2.67 (0.25-23.74)	0.36
Source						
Homebred	5	364	369 (77.3)	1		
Purchased	2	105	107 (22.7)	1.38 (0.27-7.25)	1.38 (0.17-7.55)	0.69
Body condition						
Poor	1	196	197 (41.4)	1		
Medium	3	204	207(43.5)	2.88 (0.29-27.94)	3.00 (0.27-28.38)	0.33
Good	3	69	72 (15.1)	8.52 (0.87-83.29)	8.53 (0.85-83.34)	0.02
Household TB status						
Negative	2	159	161(33.8)	1		
Positive	5	310	315(66.2)	1.28 (0.24-6.68)	1.39 (0.31-7.10)	0.76

BTB: bovine tuberculosis, n: number of total cattle tested. COR: crude odds ratio, AOR: Adjusted odds ratio, CI: confidence interval.

3.4.4. Zoonotic transmission of TB and participants' awareness

3.4.4.1. Zoonotic transmission of TB

In the present study, molecular typing of culture positive isolates using RD9-based PCR confirmed that all the human isolates were *M. tuberculosis*(Figure 13). Furthermore, no *M. bovis* was detected even from those TB patients who owned tuberculin reactor cattle. Hence, this study did not reveal evidence of direct transmission of tuberculosis from cattle to their closely associated owners.

3.4.4.2. Awarness on zoonotic transmission of TB

About 68% (74/111) of TB patientsdid not know about the transmission of TB from cattle to humans or vice versa. About 69% (77/111) of the respondentshad habit of consuming raw milk and other uncooked dairy products (Table 13).

The logistic regression, taking log-odds of awarness about zoonotic transmission as an outcome variable, resulted patient origin and educational status were observed to be significantly associated ($p<0.05$) (Table 13). The study participants in Libo kemkem and Simada were 11 and 10 times more aware about the zoonotic tramnsmission of TB as compared to those of Dera District (Libo kemkem vs. DeraAOR= 11.84; 95% CI: 1.67-73.2; p = 0.003) and (Simada vs. DeraAOR= 10.47; 95% CI: 0.72-116.3; p =0.038). The odds of having awareness on zoonotic transmission of TB was higher among individuals with secondary school educational level (AOR=4.16; 95% CI: 1.05-15.57;p = 0.029) compared to those of illiterates (Table 13). However, other patient characteristics such as age groups, sex, TB history in the family, raw milk consumption habit, patient category (new or retreatment cases) andclinical presentation of the dsease (PTB or EPTB) were not significantly associated with particpantsô over all awarness about zoonotic transmission of TB (Table 13).

Table 13. Association of demographic factors with awarness to zoonotic transmission of TB among AFB culture positive TB patients (N=111), South Gondar Zone, northwest Ethiopia (2015-2017).

Demographic factors	Number of respondents (%)	Aware of zoonotic TB		COR (95% CI)	AOR (95% CI)	p-value
		Yes ((%)	No (%)			
Patient origin						
Dera	26 (23.4)	6 (23.1)	20 (76.9)	1.00	1.00	
Ebinat	10 (9)	2 (20)	8 (80)	0. 83(0.13-5.03)	0.66 (0.39-1.28)	0.842
Este	15 (13.5)	1(6.7)	14 (93.3)	0.23(0.02-2.20)	0.25 (0.01-2,53)	0.178
Farta	9 (8.1)	2 (22.2)	7(77.8)	0.95(0.15-5. 86)	0.63 (0.02-5.53)	1.00
Fogera	35 (31.5)	13(37.1)	22(62.9)	1.96(0.62-6.16)	1.99 (0.58-8.02)	0.240
Gayint	6 (5.4)	3(50)	3(50)	3.33(0.52-21.03)	3.24 (0.31-21.0)	0.186
Libo kemkem	7(6.3)	5(71.4)	2 (28.6)	11.66(1. 89-71.79)	11.84 (1.67-73.2)	0.003
Simada	3(2.7)	2 (66.7)	1(39.3)	10.0(0. 87-114.74)	10.47(0.72-116.3)	0.038
Age group (year)						
<18	10(9)	5(50)	5(50)	1.00	1.00	
18-30	42(37.8)	17(40.5)	25(59.5)	0.68(0.17-2.71)	0.64 (0.13-2.94)	0.583
31-43	35(31.5)	8(22.9)	27(77.1)	0.29(0.06-1.28)	0.32 (0.04-1.42)	0.094
44-56	18(16.2)	5(27.8)	13(72.2)	0.38(0.07-1.92)	0.46 (0.09-2.31)	0.239
>56	6(5.4)	2(33.3)	4(66.7)	0.50(0.06-4.09)	0.51 (0.07-5.21)	0.515
Sex						
Male	58(52.3)	18(31.0)	40(69.0)	1.00	1.00	
Female	53(47.7)	19(35.8)	34(64.2)	1.24(0.56-2.73)	1.16 (0.49-2.97)	0.590
Education status						
Illiterate	73(65.8)	22(30.1)	51(69.9)	1.00	1.00	
Adult education	5(4.5)	1(0.2)	4(99.8)	0.57(0.06-5.48)	0.23 (0.04-5.53)	0.630
Primary level	18(16.2)	4(22.2)	14(77.8)	0.66(0.19-2.24)	0.62 (0.08-2.62)	0.505
Secondary level	11(9.9)	7(63.6)	4(36.4)	4.05(1.07-15.28)	4.16 (1.05-15.57)	0.029
Higher level	4(3.6)	3(0.75)	1(0.25)	6.95(0.68-70.60)	6.52 (0.62-70.71)	0.062
Raw milk consumption						
Yes	77(69.4)	27(35.1)	50(64.9)	1.00	1.00	
No	34(30.6)	10(29.4)	26 (70.6)	0.71(0.29-1.69)	0.71 (0.26-1.88)	0.441
Patient category						
New	91(82.0)	29(31.9)	62(68.1)	1.00	1.00	
Retreatment	20(18.0)	8(40.0)	12(60.0)	1.42(0.52-3. 86)	1.38 (0.42-3.93)	0.484

COR: crude odds ratio, AOR: adjusted odds ratio, CI: confidence interval.

4. DISCUSSION

The 6.3%overall prevalence of all forms of TB recorded in individuals visting public health institutions in the present studywas comparable with the report (6.2%) from northeast Ethiopia (Amare *et al*., 2013), whereas relatively lower prevalence of TB was reported among private health clinic attendees (5.4%)(Alemayehu *et al.,* 2017) and in the prison population in Gondar, northwest Ethiopia (5.3%)(Gebrecherkos *et al*., 2016).On the contrary, some studies in Ethiopiahad revealed higher prevalence of TB within the range of 7.5% to 17.3% (Mesfin *et al.,* 2005; Moges *et al.*, 2012; Wondmeneh *et al*., 2012;Tulu *et al.,*2014). The observed variations in TB prevalence might be due to differencesin the quality of diagnostic techniques used as well as variations in the living condition the study populations.

The 62.4% EPTB among the smear positive TB cases in the present study was higherthan reports of other two studies conducted in northwest Ethiopia (59.8%)(Tsegaye *et al.,* 2014) and 28.3%(Tessema *et al*., 2009).The reason for the high prevalence of EPTB in the present study might be due to the fact that many of the study participants were from referal hosptals where suspected EPTB cases from all walks of life, with diverse food habits,were diagnosed.

In this study, the higher TB prevalence found in Dera and Fogera districts might be due to high social mobility and more densly populated inhabitants (CSA, 2007) which inturn favored the transmission of the disease in these areas. In agreement with this finding, place of residence was reported as a risk factor for TB by other researchers (Amare *et al*., 2013; Gebrecherkos, *et al*., 2016).

Inthis study none of the demographic or clinical characteristics of the study participants were observed to be significantly associated with the development of EPTB. Consistently, a study in Gondar reported a non-significant association of age, sex, educational status, residence and occupational status of the respondents with EPTB infection (Zenebe *et al*., 2013). However, some studies elsewhere reported that occurence of EPTB depends on the region, ethnic background, age, underlying disease, immune status of the patient as well as genotype of the *M.tuberculosis* strain (Viedma *et al*., 2005; Forssbohm *et al*.,2008; Pareek *et al*., 2012). Such differences might be due to variationsin the use of more advanced diagnostic techniques in the previous studies than the less sensitive smear microscopy used in our study, which has been used as a routine diagnostic tool in poor resource settings such as the present study area.

The identification of *M. tuberculosis* as the only *Mycobacterium* species in the present study,using RD9-based PCR,was in agreement with previous reports in other parts of Ethiopia in which all or the majority of the isolates found from human TB cases were *M. tuberculosis* (Diriba *et al.*, 2013;Belay *et al*., 2014; Debebe *et al*., 2014; Nuru *et al*., 2015).In contrast, previous studies conducted in large scale commercial farmsand pastoral communities in lowland areas suggested the contribution of *M. bovis* to the overall burden of TBin humans (Gumi*et al*., 2012;Tibebu*et al*., 2014). The reason for the difference in *Mycobacterium* species prevalence in this study and previous studies might be due to the low TB infection rate in cattle owned by smallholder farmersthat participated in the present study.

The relatively low, 36.5% (35/96), genetic diversity of spoligotype strains in this study was consistent with similar studies conducted in Ethiopia (Mihret *et al*., 2012; Diriba *et al*., 2013; Belay *et al.,* 2014; Debebe *et al*., 2014). This might be an indication that genetic diversity of spoligotype strains is limited in Ethiopia.

The most prevalent *M. tuberculosis* strains in this study, SIT53 (lineage 4) and SIT149 (lineage 4), have been frequently reported from other parts of Ethiopia (Mihret *et al*., 2012; Firdessa *et al*.,2013; Garedew *et al*., 2013; Belay *et al*., 2014; Debebe *et al*., 2014; Korma *et al*., 2015; Nuru *et al*.,2015) while strain SIT428 (lineage 3) appears to be specific to the study area since it was not reported in previous studies from other parts of Ethiopia and East Africa. However, SIT428 was reported in some parts of Asia such as India (Desikan*et al*., 2016**)** and Iran (Mozafari *et al*., 2013). In the present study, the study area-specific SIT428 strain was isolated only from EPTB patients suggesting that this strain has an important epidemiological link, particularly to TB type.

Although Lineage 3 and 4 are found to be the abundantly distributed lineagres in South Gondar zone, other lineages were also observed to be distributed across the districts of the zone, suggesting a diversified distribution of mycobacterial lineages in the study area. Particularly, the detection of Lineage 7 in the districts of Gayint, Este, Farta and Simada, which are adjusent districts of northeastern Ethiopia (Dessie area) in which the strain was first detected in the country, further confirms the distribution of Lineage 7 in the proximal areas.

The 17.7% of the strains identified by spoligotyping, in this study,can be considered ñorphanò strains since they are new and not registered in the international spoligotype database. This observation of considerable number of ñorphanò strains was comparable to the previous reports from the same geographic area, northwest Ethiopia (Debebe *et al*., 2014; Nuru *et al*., 2015), indicating the circulation of the same spoligotype strains in the proximal areas.

In agreement with previous studies conducted in Ethiopia (Disassa *et al*., 2015; Korma *et al*., 2015; Nuru *et al*., 2015; Asebe *et al*., 2016), lineage 4 (Euro-American) was the dominant (62.5%) major lineage identified, while T3-ETH, among the most prevalent T family,constituted a considerable share (14.6%) in this study.This is in agreement with previous studies that reported a high proportion of T3-ETH from mycobacteria isolates from Ethiopia (Mihret *et al*., 2012; Belay *et al*., 2014; Esmael *et al*.,2014).

In the present study, except partly for the patientsô origin, no significant association was observed between socio-demographic factors and mycobacterial strain clustering. Similarly, studies conducted in northwest Ethiopia (Nuru *et al*., 2017) and elsewhere (Oelemann *et al*., 2007; Mathema *et al*., 2015) also reported no significant association in this respect. This lack of association may be explained by high force of interaction (proportion of susceptible individuals who have become infected in a specified period), which would result in considerable rates of TB infections (Mathema *et al*., 2015).In this study, the observed high clustering rates of *M. tuberculosis* strains in Dera (89.7%), Simada (77.8) and Fogera (72.7%) were possibly due to the relatively high population density high TB prevalence, suggesting an ongoing TB transmission in the study area.

The 18.0% overall resistance for atleast one of the two most effective first line anti-TB drugs (RIFand INH) observed was comparable to an earlier report from southwest Ethiopia (Abebe *et al*., 2012) indicating that drug resistance has not worsened in the area over the period of five years (2017). Other drug resistance reports from northeastern (17.8%) and eastern (18.4%) Ethiopia (Maru *et al*., 2015; Brhane*et al*., 2017) also show that anti-TB drug resistance levels appear to be stabilizing at a relatively high level. . However, the fact that lower resistance levels to one of the first line anti-TB drugs were reported in northern (6.7%) parts of Ethiopia (Mekonen *et al*., 2010; Biadglegne *et al*., 2013) and even a much higher level of resistance (ranging from 22-35.5%) reported from different parts of the country (Abate *et al*., 2012;Esmael *et al*., 2014; Seyoum *et al*., 2014;Ali *et al*., 2016, Bedewi *et al*., 2017) may indicate a non-uniform application of anti-TB drug policy in Ethiopia. It may also be a reflection of variable anti-TB drug pressure in the different study sites where the data were generated. This appears to be true for the study population in the present study where the majority (81.1%) of the study participants were newly diagnosed, which might lower the intensity of transmission of drug resistant TB as compared to the urban and prison settings in the previous studies where higher TB circulation and lower anti-TB drugs adherence could be noticed.

The overall MDR-TB prevalence (1.8%)and in those previously treated TB cases (9.5%) were comparable with previous studies conducted in Ethiopia (Gebeyehu *et al*., 2011; Ali *et al*., 2016; Bedewi *et al*., 2017). In contrast, higher levels of over all MDR-TB cases were revealed in Oromia Region (33%) (Mulisa *et al*., 2015) and southwest Ethiopia (27.7%) (Tadesse *et al*., 2016). Studies conducted in Uganda (14%) and Tanzania(6.3%) reported higher frequencies of MDR-TB cases among new TB cases (Lukoye *et al*., 2013; Hoza *et al*., 2015). The dfferences in overall prevalence of MDR-TB may be due to variation in the number of samples and patient selections. Since selection of thestudy participants in those studies involved majority of retreatment cases, this may have increased the number of MDR-TB cases unlike the present study in which about 81% of the study participants were newly diagnosed for TB.

The identification of complete preference of *katG* mutations without concurrent *inhA* promoter mutation suggests a high level of INH-resistnce among *M. tuberculosis* strains circulating in the study area. In agreement with the findng, a study conducted in Amhara Region reported all the INH-resistant isolates to be due to mutations at *katG* gene (Biadglegne *et al*., 2013). Higher frequencies of *katG* mutations were also reported in central Ethiopia and Oromia region (Bedewi *et al*., 2016; Tadesse *et al*., 2016; Brhane*et al*., 2017). Similarly, studies from Ghana and China had also reported higher frequencies of *katG* mutations among INH-resistant isolates, 75.8% and 92.5%, respectively (Otchere *et al*., 2016; Daoqun *et al*., 2017).

The exceptions to the specifc mutation patterns involving unusual or rare mutation pattern noticed at *katG* gene, in which both the *katG*WT and *katG*MUT types were present simultaneously,in *M. tuberculosis* isolates, signals hetroresistance condition (de Oliveira *et al*., 2003). Moreover, the WT7 probes in the MDR-TB cases may have been the *proB* gene missed without the presence of the corresponding MUT probes and hence may be considered as an ώunknownô mutation. Both rare mutation patterns detected in the study were also reported by other studies elsewhere in Ethiopia (Bedewi *et al*., 2016; Tadesse *et al*., 2016).The hetroresistant cases may be due to selection of *M. tuberculosis* isolates with random mutatons during inadequate treatment or by mixed infections with sensitive and resistant strains (Rinder *et al*., 2001).Such phenomenon is known to be matters of serious concern as it will jeoparadize the effective treatment of patients with INH, thereby leading to the development of anti-TB drug resistance, treatment failure or relapse in the study area (Hofmann-Thiel *et al*., 2009).

Consistent with the present finding, a higher frequency of any drug resistance to first line anti-TB drug, among Euro-American lineages of *M. tuberculosis* was reported in central Ethiopia (Bedewi *et al*., 2017). Although the association was not statistically significant, the observed highest proportion of drug resistant cases among the T-sublineage detected in this study was similar to studies from other African countries - Tanzania and Uganda (Kibiki *et al*., 2007; Bazira *et al*., 2011).The higher frequency of any anti-TB drug resistance in the clustered strains is suggestive of the higher risk of drug resistance in recent TB transmission in the study area.

In agreement with the prevalence report of this study, low prevalence of bovine TB was also reported from Yeki District, southern Ethiopia and around Bahir Dar City in the northwest (Admasu*et al*., 2014; Nuru *et al*., 2015). On the other hand, higher prevalence of bovine TB was reported in and around other cities of Ethiopia (Ameni and Aklilu, 2007; Ameni *et al*., 2008; Regassa *et al*., 2008; Tsegaye*et al*., 2010; Firdessa*et al*., 2012). These variations in the prevalence of bovine TB are associated with the breed of cattle kept and the type of husbandry under which the cattle are kept. Previous studies in Ethiopia have indicated that *Bos tauruss* breed is more susceptible to bovine TB as compared to *Bos indicus* breed (Ameni *et al*., 2006). Moreover, it was observed that cattle kept in intensive farms are more susceptible to bovine TB as compared to cattle kept in extensive farms (Ameni *et al*., 2006). In addition, it was well established that the prevalence of bovine TB is directly associated with the herd size. Thus, the observation of low prevalence of bovine TB in the present study is not surprising as all the study cattle were *Bos indicus* and were also kept in extensive farming; both of which do not favour the occurrence and transmission of bovine TB.

Although zoonotic transmission of *M. bovis* from cattle to farmers was expected in the present study, all the human isolates were *M. tuberculosis*. Nevertheless, previous study conducted in and around Bahir Dar City (Nuru *et al*., 2017) and Borena Zone (Gumi *et al*., 2012) reported the isolation of *M. bovis* from human TB cases. However, since it has been shown (Cosivi *et al*., 1998) that the prevalence of human TB, caused by *M. bovis* in specific geographic region, is directly proportional to the prevalence of bovine TB in that specific geographic region, the prevalence of bovine TB was very low and hence the chances of its transmission to humans was minimal.

Nevertheless, although the overall prevalence of bovine TB recorded by the present study was low, it was relatively higher in cattle owned by TB positive households than cattle owned by TB free households, suggesting that zoonotic transmission cannot be ruledout in the study area. This suggestion has support from earlier studies that reported higher prevalence of bovine TB in cattle owned by households with active TB cases than TB free households (Regassa*et al*., 2008; Ameni*et al*., 2013; Tamiru *et al*., 2013; Mengistu *et al*., 2015). Since diagnosis of TB in the cattle was performed by SIDCTT and positivity to SIDCTT might be also due to sensitization to infection with *M. tuberculosis* as it was observed earlier by other authors (Berg *et al*., 2009; Ameni*et al*., 2011; Ameni*et al*., 2013), this would imply that *M. tuberculosis* might have been transmitted to cattle from their owners.

5. CONCLUSIONS

A high prevalence (6.3%) of all forms of TB was observed in South Gondar Zone, northwest Ethiopia, for which different socio-demographic risk factors may have been contributing.

Of the total TB cases detected in the study area, EPTB was more common (62.4%) than PTB.

The observed higher clustering rate (72.9%) and the identified óorphanô strains of *M. tuberculosis* isolates indicated a higher rate of recent and ongoing transmission of *M. tuberculosis* strains in the study area.

A considerably high proportion (18.0%), of *M. tuberculosis* strains were found to be resistant to at least one of the two most effective first line anti-TB drugs, INH and RIF, with MDR-TB detected in 1.8% of the cases.

Although the present study did not reveal transmission of TB from cattle to farmers, the presence of tuberculin reactive cattle (1.6%) owned by TB patients coupled with high raw milk consumption habit (69.4%) and the low (33%) level of awareness towards zoonotic transmission of TB, suggested the public health risk of zoonotic TB in South Gondar Zone.

Limitations of the study

1. The present study did not address the impact of other factors such as HIV/AIDS on TB prevalene and the development of drug resistant TB in the study area.
2. The study revealed the genetic diversity of *M. tuberculosis* and the presence of orphan (new) strains solely based on spoligotyping data.

3. No pathological examination and mycobacterial culture was performed for tuberculin reactive cattle.
4. Current approaches of genotyping (MIRU-VNTR and Whole Genome Sequencing (WGS) could not be performed).

6. RECOMMENDATIONS

1. The observed high prevalence of human TB, high clustering of *M. tuberculosis* strains and considerable proportions of drug resistant-TB suggest the need to strengthen the preventive and control efforts of the disease in the study area.
2. Proper surveillance of mycobacterial isolates supported by use of molecular typing methods, with better discriminatory power than spoligotyping, is necessary to control the transmission of TB in the study area.
3. Further investigation involving post-mortem pathological examination and molecular characterization of mycobacterial isolates from tuberculin reactor cattle should be done to complement the findings of the present study.
4. Awarness creation and education about the possible risk of zoonotic transmission of TB would be a valuable additional measure for TB control inthe study area.

REFERENCES

Abate, D., Taye, B., Abseno, M. and Biadgilign, S. (2012). Epidemiology of anti-tuberculosis drug resistance patterns and trends in tuberculosis referral hospital in Addis Ababa, Ethiopia. *BMC Res. Notes* **5**:462.

Abebe, G., Abdissa, K., Abdissa, A., Apers, L., Agonafir, M., de Jong, B.C., *et al*. (2012). Relativelylow primary drug resistant tuberculosis in south western Ethiopia.*BMC Res.Notes* **5**:225.

Addis, Z., Adem, E., Alemu, A., Birhan, W.,Mathewos, B., Tachebele,B.,*et al*. (2015) Prevalence of smear positive pulmonary tuberculosis in Gondar prisoners north west Ethiopia.*Asian Pac. J. Trop. Dis.***8**(**2**):127-131.

Admasu, P., Berihun, W. And Niguse, A. (2014). Prevalence of Bovine Tuberculosis in Dairy Cattle of Yeki District, Southern Ethiopia. *AJBAS.* **6 (5):**135-140.

Alemayehu, M., Tigabu, A., Yunkura, S., Hagos, F. and Tegene, B. (2017). Prevalence of Smear Positive Pulmonary Tuberculosis and Associated Risk Fact ors among Pulmonary Tuberculosis Suspected Patients at Private Health Institu tions in Gondar Town Northwest Ethiopia: A Cross Sectional *Am. J. Infect. Dis .Micrbiol.***5 (1)**: 61-65.

Alemayehu, M., Gelaw, B., Abate, E.,Wassie, L.,Belyhun, Y.,Bekele, S., *et al*. (2014) Active tuberculosis case finding and detection of drug resistance among HIV i nfected patients: A cross sectional study in a TB endemic area, Gondar, Northw est Ethiopia. *Int. J. Mycobacteriol.***3**: 132 ï 138.

Alexander, A., Laver, N., Michel, L.,Williams, M, van Helden, D., Warren, M., *et al*. (2010). Novel *M. tuberculosis* complex pathogen, *M. mungi*. *Emerg. Infect. Dis.***16**, 1296-1299.

Ali, Beckert, P., Haileamlak, A., Wieser, A., Pritsch, M., Heinrich, N., *et al.* (2016). Drug resistance and population structure of *M. tuberculosis* isolates from prison -s and communities in Ethiopia. *BMC Infect. Dis.***16**:687.

Ali, A., Hasan, R., Jabeen, K., Jabeen, N., Qadeer, E. and Hasan, Z. (2011).Characterization of Mutations Conferring Extensive Drug Resistance to *M. tuberculosis* Isolates in Pakistan. *Antimicrob. Agents Chemother.* **55**(**12**): 5654ï 5659.

Amare, H., Gelaw, A., Anagaw, B. and Gelaw, B. (2013). Smear positive pulmonary tuberculosis among diabetic patients at the Dessie referral hospital, northeast Ethiopia. *Infect. Dis. Poverty***2**:6.

Ameni, G., Regassa, A., Kassa, T. and Medhin, G. (2001). Survey on bovine tuberculosis and its public implications to cattle raising families in Soddo, Southern Ethiopia. *EJAP.***1(1):**55- 62.

Ameni,G., Aseffa, A.,Engers, H., Young, D.,Hewinson, G and Vordermeier,M.(2006). Cattle Husbandry in Ethiopia Is a Predominant Factor Affecting the Pathology of Bovine Tuberculosis and Gamma Interferon Responses to Mycobacterial Antigens. *Clin. Vaccine Immunol.* **13(9):**1030-1036.

Ameni, G., Aseffa, A., Engers, H., Young, D., Gordons, S., Hewinson,G. *et al*. (2007) High prevalence and increased severity of pathology of bovine tuberculosis in Holsteins compared to zebu breeds under fiel cattle husbandryin central Ethiopia. *Clin. Vaccine Immunol.***14:**1356-1361.

Ameni, G. and Erkihun, A. (2007). Bovine tuberculosis on small scale dairy farms and farmer awareness of the disease in Adama Town, central Ethiopia. *Rev. Sci. Tech.* OIE, **26 (3):** 711-719.

Ameni, G., Hewinson, G., Aseffa, A., Young, D. and Vordermeier, M. (2008).Appraisal of interpretation criteria for the comparative intradermal tuberculin test for th e diagnosis of bovine tuberculosis in Central Ethiopia. *Clin.Vaccine Immunol.***1 5:**1272ï 1276.

Ameni, G., Bekele, S., Tolosa, T. (2010a). Preliminary study on the impact of bovine tuberculosis on the reproductive efficiency and productivity of Holstein dairy cows incentralEthiopia.*Bulleti Anim Health Prod Africa* **58:** 222ï 226.

Ameni, G., Tadesse, K., Hailu, E., Deresse, Y., Medhin, G., Aseffa, A., *et al*. (2013). Transmission of *Mycobacterium tuberculosis* between Farmers and Cattle in Central Ethiopia.https://doi.org/10.1371/journal.pone.0076 891.

Ameni, G., Vordermeierb, M., Firdessa, R., Aseffa, A., Hewinson, G., Gordonbde, S., *et al*. (2011). *Mycobacterium tuberculosis* infection in grazing cattle in central Ethiopia.*Vet. J.***188:** 359-361.

Angesom,D.(2016).Community Based Intervention for Zoonotic Diseases Preventionand Control in Ethiopian Pastoral Areas. *J.Pharm. Alt.Med.* **(10):** 2222-4807.

Annika, K. (2016). Forest Cover Change in South Gondar, Ethiopia from 1985 to 2015: Landsat Remote Sensing Analysis and Conservation Implications: **pp**.4-7.

Asebe, G., Worku, A., Zewude, A., Tafess, K. and Ameni, G. (2016). Molecular characterizat-ion of *Mycobacterium tuberculosis* complex in Gambella region,Southwest Ethiopia.*Afri. J. Microbiol. Res.***10**(**29**): 1086-1093.

Ashford, A., Whitney, E., Raghunathan, P. and Cosivi, O. (2001). Epidemiology of selected mycobacteria that infect humans and other animals. *Rev. Sci.Tech.* (International Office of Epizootics)**20(1):**325-337.

Awulachew,B., McCartney, M., Steenhuis, T. and Ahmed, A. (2009). A Review of Hydrology, Sediment and Water Resource Use in the Blue Nile Basin.International Water Management Institute, Colombo, Sri Lanka.

Ayaz, A., Hasan, Z., Jafri, S., Inayat, R., Mangi, R., Channa, A., *et al*. (2012). Characterizing *Mycobacterium tuberculosis* isolates from Karachi, Pakistan: drug resistance and genotypes. *Int. J. Infect. Dis.***16:** e303ï e309.

Ayele, Y., Neill, D., Zinsstag, J., Weiss, G. and Pavlik, I. (2004).Bovine tuberculosis: an old disease but a new threat to Africa.*Int. J. Tuberc Lung Dis.***8:** 924-937

Azage, T., Berhanu, G. and Dirk, H. (2013). Smallholder Dairy Production and Marketig Systems in Ethiopia: IPMS Experiences and Opportunities for Market Oriented Development. International Livestock Research Institute (ILRI), Nairobi.

Barco, P., Cardoso, F., Hirata, D., Leite, Q., Pandolfi, R., Sato, N., *et al.* (2006). A mutations in pyrazinamide resistant *Mycobacterium tuberculosis* clinical isolates from the southeast region of Brazil. *J. Antimicrob. Chemother.***58:** 930-935.

Bazira, J.,Asiimwe, B., Joloba, M., Bwanga, F.and Matee, M. (2011). *Mycobacterium tuberculosis* spoligotypes and drug susceptibility pattern of isolates from tuberculosis patients in South-Western Uganda. *BMC Infect. Dis.***11**:81.

Bedewi, Z., Mekonnen, Y., Worku, A., Medhin, G., Zewde, A.,Yimer,G., *et al.*(2017) *Mycobacterium tuberculosis* in central Ethiopia: drug sensitivity patternsand association with genotype. *New Microbes New Infect.***17:** 69ï 74.

Bedewi, Z.O., Mekonnen, Y.,Worku, A.,Zewde, A., Medhin, G., Mohammed, T., *et al* (2016). Evaluation of the GenoType MTBDRplus assay fordetection of rifampicin- and isoniazid-resistant*Mycobacterium tuberculosis* isolates in centralEthiopia. *Int. J.Mycobacteriol*.**5:** 475-481.

Belay, M., Ameni, G., Bjune, G., Couvin, D., Rastogi, N. and Abebe, F. (2014).Strain diversity of *Mycobacterium tuberculosis* isolates from pulmonary tuberculosis patients in Afar pastoral region of Ethiopia. *BioMed. Res. Inter.*, http://dx.doi.or g/10.1155/2014/.

Belay, D., Yisehak, K. and Janssens, G. (2012). Survey of major diseases affecting da iry cattle in Jimma town, Oromia, Ethiopia.*Global Vet.***8:** 62-66.

Berg, S., Firdessa, R., Habtamu, M., Gadisa, E., Mengistu, A. and Yamuah, L. (2009). The burden of mycobacterial disease in Ethiopian cattle: implications for public health. *PLoS One,* **4:**e5068.

Berg, S., Schelling, E., Hailu, E, Firdessa, R., Gumi, B., Erenso, G., *et al.* (2015). Investigation of the high rates of extrapulmonary tuberculosis in Ethiopia reveals no single driving factor and minimal evidence for zoonotic transmission of *Mycobacterium bovis* infection. *BMC Infect. Dis*.**15:** 112.

Bertu, J., Depar, M., Gusi, M., Ngulukun, S., Leo, S. and Jwander, D. (2010). Prevalence of brucella antibodies in marketed milk in Jos and environs.*AJFS.***4**: 62-64.

Biadglegne, F., Tessema, B., Sack, U. and Rodloff, A. (2014). Drug resistance of *Myc obacter-ium tuberculosis* isolates fromtuberculosis lymphadenitis patients in Ethiopia. *Indian J. Med. Res.***140:**116-122.

Biadglegne, F., Tessema, B., Rodloff, A. and Sack, U. (2013). Magnitude of Gene Mu tations Conferring Drug Resistance in *Mycobacterium tuberculosis* Isolates fro m Lymph Node Aspirates in Ethiopia. *Int. J. Med. Sci.*1**0(11):**1589-1594.

Bifani, J., Plikaytis, B., Kapur, V., Stockbauer, K., Pan, X., Lutfey, L., *et al*. (1999). Origin and interstate spread of a New York city multidrugresistant *Mycobacteri um tuberculosis* clone family. *JAMA*.**275**:452-457.

Bhatt, A., Molle, V.,Besra, G., Jacobs, W. and Kremer, L. (2007).The *Mycobacteriu tuberculosis* FASII condensing enzymes: their role in mycolic acid biosynthesis acid-fastness, pathogenesis and in future drug development. *Mol. Microbiol.***64 (6):** 1442ï 1454

Brhane, M., Kebede, A. and Petros, Y. (2017). Molecular detection of multidrug-resistant tuberculosis among smear positive pulmonary tuberculosis patients in Jigjiga town, Ethiopia.*Infect. Drug Resist.***10:** 75ï 83.

Brosch, R., Gordon, S. V., Marmiesse, M., Brodin, P., Buchrieser, C., Eiglmeier, K., *et al*. (2002).A new evolutionary scenario for the Mycobacterium tuberculosis complex.*Proc. Natl. Acad. Sci.***99:**3684ï 3689.

Brudey, K., Driscoll, R., Rigouts, L.,Prodinger, M.,Gori, A.,Al Hajoj, A., *et al***.** (2006) *Mycobacterium tuberculosis* complex genetic diversity: mining the fourth inter national spoligotyping database (SpolDB4) for classification, population geneti cs and epidemiology. *BMC.Microbiol.***6**:23-50.

CDC (2011).*Mycobacterium bovis* (bovine tuberculosis) in humans.US Centers for Disease Control and Prevention. http://www.cdc.gov/tb/ publications/factsheets /general/mbovis.pdf (accessed Dec 6, 2016).

Cheng, J., Thibert, L., Sanchez, T., Heifets, L. and Zhang, Y. (2000).*pncA* mutations as a major mechanism of pyrazinamide resistance in *Mycobacterium tuberculosis*: spread of a mono resistant strain in Quebec, Canada.*Antimicrob.Agents Chemother.***44:**528ï 32.

Chevalier, F.,Cascioferro, A.,Majlessi, L.Herrmann, J. and Brosch, R. *(*2014). *Micob acterum tuberculosis* Evolutionary Pathogenesis and its Putative Impact on Dr ug Development.Future *Microbiol.***9**(**8**):969-985.

Cosivi, O., Grange, M., Daborn, J., Raviglione, C., Fujikura,T.and Cousins, D.(1998). Zoonotic tuberculosis due to *Mycobacterium bovis* in developing countries. *Emerg. Infect. Dis.***4:**59-70.

Cleaveland, S., Shaw, J., Mfinanga, G.,Shirima, G., Kazwala, R. and Eblate, E.(2007). *M.bovis* in rural Tanzania: risk factors for infection in human and cattle popula tions.*Tuberculosis.* **87:** 30-43.

Cole, T., Brosch, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D., *et al*. (1998). D eciphering the biology of *Mycobacterium tuberculosis* from the complete geno me sequence. *Nature,***393**:537ï 544.

Crofton, J. and Mitchison, A. (1948).Streptomycin resistance in pulmonary tuberculosis.*Br. Med. J.* **2:**1009-15.

CSA. (2017). Federal Democratic Republic of Ethioia Central Statistics Agency: Agricultural Sample Survey 2016/17 [2009 E.C]: Volume II, Report on Livestock and Livestock Characteristics (Private Peasant Holdings). Addis Ababa, Ethiopia. Available at: www.csa.gov.et/. Accessed date [22/01/18].

CSA. (2007). Summary and Statistical Report of the 2007 Population and Housing Ce nsus: Population Size byAge andSex. Central Statistics Agency,Addis Ababa, Ethiopia.

Daoqun, L., Yuzhu, S., Cheng-Lin, Z., Xiaofei, L., Xueshan, X. and A-Mei, Z. (2017). Screening mutations in drug-resistant *Mycobacterium tuberculosis*strains in Yunnan, China.*J Infect. Public Health***10**: 630ï 636.

Davies, P. (2006). Tuberculosis in humans and animals: are we a threat to each other? *J. Roy. Soc.Med.***99**(**10**): 539-540.

Debebe, T.,Admassu, A.,Mamo, G. And Ameni, G. (2014).Molecular characterization of *Mycobacterium tuberculosis* isolated from pulmonary tuberculosis patients i n Felege Hiwot Referral Hospital, northwest Ethiopia. *J.Microbiol. Immunol. Infect.***47:**333- 338.

De Oliveira, M., de Silva, A., Cardoso, M. (2003).Rapid detection of resistance against rifampicin in isolates of *Mycobacterium tuberculosis*from Brazilian patients using a reverse-phase hybridization assay.*J. Microb. Methods***53**: 335-342.

De Rossi, E., Aĕnsa, J.A. and Riccardi, G. (2006). Role of mycobacterial effluxtransp orters in drug resistance: an unresolved question. *Microbiol.Rev.***30:** 36ï 52.

Desikan, P., Chauhan, D., Sharma, P., Panwalkar, N., Chourey, M., Patidar, M.,*et al*. (2016). Genetic diversity of *Mycobacterium tuberculosis* isolates from central India.*Indian J Med. Res.***143:** 481-486.

Diriba, B., Berkessa, T., Mamo, G., Tedla, Y. and Ameni, G. (2013). Spoligotyping of multid-rug-resistant *Mycobacteriumtuberculosis* isolates in Ethiopia. *Int. J Tuberc Lung Dis.***17**(**2**)**:**246ï 250.

Disassa, H., Tafess, K.,Worku, A.and Ameni, G. (2015). A Preliminary Study on Molecular Characterization of *Mycobacterium tuberculosis* in Benishangul Gu muz Region,Western Ethiopia. *Br. Microbiol. Res. J.* **10(6):** 1-10.

Duguma, B. and Janssens, G.(2015). Assessment of Dairy Farmersô Hygienic Milking Practices and Awareness of Cattle and Milk-Borne Zoonoses in Jimma, Ethiopia. *Food Sci. Qual. Manag.***45:** 2224-6088.

EDHS. (2011). Ethiopia Demographic and Health Survey: Central Statistical Agency, Addis Ababa, Ethiopia and ICF International Calverton, Maryland, USA.

Eliso, E., Medhin, G. and Belay, M. (2015). Prevalence of smear positive pulmonary t uberculosis among outpatients presenting with cough of any duration in Shashogo Woreda, Southern Ethiopia.*BMC Public Health* **15**:112.

Esmael, A., Ali, I., Agonafir, M., Endris, M., Getahun, M., Yarega, Z., *et al*. (2014). Drug Resistance Pattern o*f Mycobacterium tuberculosis* in Eastern Amhara Reg ionalState, Ethiopia*.Microb.Biochem. Technol.* **6:**2.

Etter, E., Donado, P., Jori, F., Caron, A., Goutard, F., Roger, F. *(*2006).Risk analysis andbovine tuberculosis, a re-emerging zoonosis.*Ann. N Y Acad. Sci.***1081:** 61ï 73.

FDRE. (2015). Federal democratic Republic of Ethiopia. Annual Tuberculosis and Le prosy Bulletin.

Firdessa, R., Tschopp, R., Wubete, A., Sombo, M., Hailu, E., Erenso, G., *et al*. (2012). High prevalence of bovine tuberculosis in dairy cattle in central Ethiopia: implications for the dairy industry and public health. *PLoS One***7 (12)**, e52851. .

Firdessa, R., Berg, S., Hailu, E., Schelling, E., Gumi, B., Erenso, G., *et al* (2013). My cobacterial lineages causing pulmonary and extrapulmonary Tuberculosis, Ethi opia.*Emerg. Infect. Dis.***19**(**3**)**:** 460ï 463.

Filliol, I., Driscoll, R., van Kreiswirth, S., Kremer, B., Valetudie, K., Anh, G., *et al*. (2002). Global distribution of *Mycobacterium tuberculosis* spoligotypes.*Emerg. Infect. Dis.* **8:**1347-1349.

Fleischmann, D.,Alland, D.,Eisen, A.,Carpenter,L.,White, O.,Peterson, J.,*et al*.(2002). Whole-genome comparison of *Mycobacterium tuberculosis* clinical and laboratory strains. *J. Bacteriol.***184** (**19**)**:** 5479-5490.

FMOH (2005).Accelerated Expansion of Primary Health Coverage in Ethiopia, Federal ministry of Health Addis Ababa, Ethiopia.

FMOH (2007b).Health Extension Program in Ethiopia Profile.Federal ministry of Health Addis Ababa, Ethiopia.

FMoH. (2008). Federal Ministry of Health, Tuberculosis, Leprosy and TB/HIV Prevention and Control Programme.Addis Ababa, Ethiopia, 4th edition.

FMoH. (2011). The first Ethiopian national population based TB prevalence survey. Federal Ministry of Health, Addis Aibaba, Ethiopia.

Fochsen, G., Deshpande K., Ringsberg, K.C., Thorson, A. (2009). Conflicting account abilities: Doctorꞌs dilemma in TB control in rural India. *Health Policy***89**: 160-167.

Forssbohm, M., Zwahlen, M., Loddenkemper, R., Rieder, L. (2008). Demographic ch aracteristics of patients with extrapulmonary tuberculosis in Germany. *Europ. Resp. J.* **31**: 99-105.

Frieden, R., Sterling, T., Pablos Mendez, A., Kilburn, O., Cauthen, M., Dooley, W. (1 993). The emergence of drug-resistant tuberculosisin New York City. *N. Eng. J. Med.***328:** 521-6.

Frieden, T.R., Lerner, B.H., Rutherford, B.R. (2000). Lessons from the 1800s: tuberculosis control in the new millennium. *Lancet***355(9209):**1088-1092.

Gafirita, J., Umubyeyi, A., Asiimwe, B. (2012). A first insight into the genotypic dive rsity of*Mycobacterium tuberculosis* from Rwanda.*BMC Clin.Pathol.* **12**:20.

Gagneux, S., DeRiemer, K., Van, T., Kato-Maeda, M., de Jong, C., Narayanan, S.,*et al*. (2006).Variable host-pathogen compatibility in *Mycobacterium tuberculosis*. *Proc. Natl. Acad. Sci.***103:**2869-2873.

Gagneux, S.,Small, M. (2007).Global phylogeography of *Mycobacterium tuberculosis* and implications for tuberculosis product development. *Lancet Infect. Dis.***7:**328-337.

Galagan, J. E. (2014). Genomic insights into tuberculosis.*Nature Rev. Gen.***15**: 307ï 320.doi: 10.1038/nrg3664

Garedew, L.,Mihret, A., Mamo, G., Abebe, T., Firdessa, R., Bekeleet, Y.,*et al*. (2013). Strain diversity of mycobacteria isolated from pulmonary tuberculosis patients at Debre Birhan Hospital, Ethiopia.*Int. J Tuberc. Lung Dis.***17**(**8**)**:**1076ï 1081.

Garnier, T., Eiglmeier, K., Camus, J.C., Medina, N., Mansoor, H., Pryor, M. *et al*. (2003).The complete genome sequence of *Mycobacterium bovis*ò.*Proc. Natl. Acad. Sci.***100(13):** 7877-7882.

Gebeyehu, M., Lemma, E., Eyob, G. (2011). Prevalence of drug resistant tuberculosisi n Arsi Zone, Ethiopia.*Ethiop. J.Health Dev.***15:**11ï 6.

Gebrecherkos, T., Gelaw, B., Tessema, B. (2016). Smear positive pulmonary tubercul osis and HIV coinfection in prison settings of NorthGondar Zone,Northwest Ethiopia.*BMC Public Health***16:**1091.

Gebreegziabher, S.,Yimer, S.,Bjune, G. (2016). Qualitative Assessment ofChallenges inTuberculosis Control in West Gojjam Zone, northwest Ethiopia:HealthWorkers and Tuberculosis Control Program CoordinatorsôPers pectives.*Tuberc.Res.* Available at: https://www.hindawi.com.

Gebremedhin, G., Sintayehu, F., Mequanente, A., Dinda, S. (2014). Anti-tuberculosis drug resistance in Ethiopia: A mata- analysis. *J.Drug Deliv. Ther.***4**(**3**): 154-163.

Getachew, F. (2003).A review of small scale dairy sector in Ethiopia.FAO prevention of food losses programme.Milk and milk products.Post-harvest losses and food safety in Sub-Saharan Africa and Near East.

Gillah, A., Kifaro, C., Madsen, J. (2012). Urban and Peri Urban Dairy Farming in East Africa: A Review on Production Levels, Constraints and Opportunities. *L RRD.***24:**11.http://www.lrrd.cipav.org.co.

Gillespie, H.(2007). Tuberculosis: evolution in millennia and minutes. *Biochem. Soc. Trans.***35**: 1317ï 20.

Gillespie, H., Dickens, A., McHugh, D. (2000). False molecular clusters due to nonra ndassociation of IS6110 with *Mycobacterium tuberculosis*. *J. Clin. Microbiol.* **38** (**6**)**:**2081-2086.

Gizaw, S., Abera, M., Muluye, M., Aliy, M., Alemayehu, K., Tegegne, A. (2017). Val idating the Classification of Smallholder Dairy Farming Systems Based on Herd Genetic Structure and Access to Breeding Services. *Agri. Sci.***8:** 545-558.

Godreuil,S.,Torrea, G.,Terru, D., Chevenet, F., Diagbouga, S., Supply, P., *et al*. (2007). First molecular epidemiology study of *Mycobacterium tuberculosis* in Burkina Faso.*J. Clin.Microbiol.***45:**921-927.

Gumi, B., Schelling, E., Firdessa, R., Aseffa, A., Tschopp, R., Yamuah, L., Young, D., Zinsstag, J., (2011). Prevalence of bovine tuberculosis in pastoral cattle herds in the Oromia region, southern Ethiopia.*Trop. Anim Health Prod.***43:**1081ï 1087.

Gumi, B.,Schelling, E., Berg, S., Firdessa, R.,Erenso, G., Mekonnen, W.,*et al*. (2012). Zoonotic Transmission of Tuberculosis between Pastoralists and Their Livestock in South-East Ethiopia.*Eco. Health***9:**139ï 149.

Hadush, D. A. (2016).Community Based Intervention for Zoonotic Diseases Prevention and Control in Ethiopian Pastoral Areas.*J. Pharm. Alt.Med.* **10:**2222-4807.

Hailemariam, S. (1975). A Brief Analysis of the Activities of the Meat Inspection and Quarantine Division. Ministry of Agriculture, Addis Ababa, Ethiopia.

Hamusse,S., Teshome,D., Hussen,M., Demissie,M., Lindtjørn,B. (2016).Primary and secondary antituberculosis drug resistance in Hitossa District of Arsi Zone, Oromia Regional State, CentralEthiopia.*BMC PublicHealth***16**:593.

Hesseling, A., Cotton, M.,Jennings, T.,Whitelaw, A.,Johnson, L.,Eley,B.,*et al.* (2009). High incidence of tuberculosis among HIV-infected infants: evidence from a South African population-based study highlights the need for improved tuberculosis control strategies. *Clin. Infect. Dis.***48**: 108-114.

Hirsh, E., Tsolaki, G., De Riemer, K., Feldman, W., Small, M. (2004). Stable association between strains of *Mycobacterium tuberculosis* and their human host populations.*Proc. Natl. Acad. Sci.***101**:4871-4876.

Hofmann-Thiel, S., van Ingen, J., Feldmann, K., Turaev, L., Uzakova, T., Murmusaeva, G. et al. (2009). Mechanisms of hetroresistance to isoniazid and rifampicine of *Mycobacterium tuberculosis* in Tashkent, Uzbekistan.*Eur. Respir. J.* **33**: 368-374.

Hoza,A.,Mfinanga, S., K¨onig, B. (2015).Anti TB drug resistance in Tanga, Tanzania: A cross sectional facility-base prevalence among pulmonary TB patients.*Asian Pac. J. Trop.Med.* **8(11)**: 907ï 913.

Huy, N., Lucie, C., Hoa, T., Van Hung, N., Lan, N., Son,N., *et al*. (2017). Molecular analysis of pyrazinamide resistance inMycobacterium tuberculosis in Vietnam highlights the high rate of pyrazinamide resistance-associatedmutations in clinical isolates. *Emerg.Microb. Infect.***6**: e86; doi:10.1038/emi.

Jure´en, P., Werngren, J., Toro, J.C., Hoffner, S. (2008). Pyrazinamide resistance and pncA gene mutations in *Mycobacterium tuberculosis*. *Antimicrob.Agents Chemother.***52**: 1852ï 4.

Kamerbeek, J.,Schouls, L.,Kolk, A., Van Agterveld, M., Van Soolingen, D., Kuijper, S.,*et al*. (1997). Simultaneous detection and strain differentiation of *M. tubercul osis*for diagnosis and epidemiology.*J Clin.Microbiol.***35** (4): 907ï 914.

Kapata,N., Mbulo,G., Cobelens,F., Haas,P., Schaap,A., Mwamba,P.,*et al*. (2015).The Second Zambian National Tuberculosis Drug Resistancesurveya comparison of conventional and molecular methods. *Trop.Med.Int. Health*. **20**(**11**):1492ï 1500.

Kapur, V., Whittam, S., Musser, M. (2000). Is *Mycobacterium tuberculosis* 15,000 Years Old. *J. Infect. Dis.***170** (**5**): 1348-1349.

Kaur, D.,Guerin, E.,Skovierova, H., Brennan, J., Jackson, M. (2009). Biogenesis of the cell wall and other glycol conjugates of *M. tuberculosis*. *Adv.Appl. Microbi ol.* **69**: 23ï 78.

Kibiki,G., Mulder,B., Dolmans,W., de Beer, J., Boeree, M., Sam,N.,*et al*. (2007).*M. t uberculosis* genotypic diversity and drug susceptibility pattern in HIV-infected and non HIV infected patients in northern Tanzania. *BMC Microbiol.***7** :51.

Kiros, T. (1998).Epidemiology and zoonotic importance of bovine tuberculosis in selected sites of Eastern Shewa Ethiopia. MSc. *Thesis.* Faculty of Veterinary M edicine, Addis Ababa, University and Freie Universitat, Berlin, Germany.

Kleeberg, H.H. (1984). Human Tuberculosis of Bovine Origin in Relation to Public Health. *Rev. Sci. Tech. OIE*.**3**: 11-32.

Korma, W, Mihret, A., Hussien, J., Anthony, R., Lakew, M., Aseffa, A. (2015). Clinical, molecular and drug sensitivitypattern of mycobacterial isolates fromextra-pulmonary tuberculosis cases in Addis Ababa, Ethiopia. *BMC Infect.Dis.***15**:456.

Kudi, A.C., Bello, A., Ndukum, J.A. (2012). Prevalence of bovine tuberculosis in camels in Northern Nigeria.*J.Camel Pract. Res.***19:** 81ï 86.

Lee, S., Othman, N., Ho, M., Wong, Y. (2004). Novel mutations within the *embB* gene in ethambutol-susceptible clinical isolates of *Mycobacterium tuberculosis*. *Antimicrob.Agents Chemother.***48**(**11**): 4447-9.

Lemma, E., Alvarez, V., Gebre Tsadik, G. (1984). Drug sensitivity patterns of *M. tuberculosis* isolates in Addis Ababa. *Ethiop. Med. J.***22**: 93-96.

Lemma, E., Niemi, M., Lynton, B., Dubrie, G. (1989). Bacteriological studies of tuberculosis in Sidamo Regional Hospital. *Ethiop. Med. J.***27**:147-149.

Li, J., Chung, P.H., Leung, C.,. Nishikiori, N., Chan, E., Yeoh E.K (2017). The strategic framework of tuberculosis control and prevention in the elderly: a scoping review towards End TB targets. *Infect. Dis. Poverty*.**6**:70

LoBue, A., Betacourt, W., Peter, C. and Moser, S. (2003). Epidemiology of *Mycobact erium bovis* disease in San Diego County, 1994ï 2000. *Int. J. Tuberc. Lung Dis.***7**: 180ï 185.

Lukoye,D., Adatu,F., Musisi,K., Kasule,G., Were,W., Odeke,R.,*et al.* (2013). Anti-Tuberculosis Drug Resistance among New andPreviously Treated

Sputum. Smear Positive Tuberculosis Patients in Uganda: Results of the First National Survey. *PLoS ONE,* **8**(**8**):e70763. doi:10.1371/journal.pone.0070763.

Mahler, H. (2007). The tuberculosis programme in the developing countries. 1966. *Bullettine,* World Health Organization, **85** (5):415-417.

Marais, B.J. (2013). History of tuberculosis and drug resistance.*N Engl. J Med.***368**(1); 88-90.

Maru, M., Mariam, S., Airgecho, T.,Gadissa, E., Aseffa, A. (2015).Prevalence of Tuberculosis, Drug Susceptibility Testing, and Genotyping of Mycobacterial Isolates from Pulmonary Tuberculosis Patientsin Dessie, Ethiopia. *Tuberc. Res.Treat.*Available at: http://dx.doi.org/10.1155/2015/215015.

Mathema, B., Kurepina, N., Bifani, P., Kreiswrith, B. (2006). Molecularepidemiology of tuberculosis current insights.*Clin.Microbiol. Rev.***19:** 658ï 685.

Mathema, B., Lewis, J., Connors, J., Chihot, V., Shashkina, E., Meulen, M., *et al*. (2015). Molecular Epidemiology of *Mycobacterium tuberculosis* among South African Gold Miners.*Anna. Am. Thor. Soc.***12** (**1**): 12ï 20.

Mathuria,J.,Srivastavab,G.,Sharmac,P.,Mathuriad,B., Ojhaa,S., Katoch,V.*et al*. (2017). Prevalence of *Mycobacteriumtuberculosis* Beijing genotype and itsassociation with drug resistance in NorthIndia.*J. Infect. Public Health***10**: 409-414.

Mawak, J., Gomwalk, N., Bello, C., Kandakai-Olukemi, Y. **(**2006). Human pulmonary infections with bovine and environment (atypical) mycobacteria in Jos, Nigeria.*Ghanaean Med. J.***40 (4):**132-136.

Melaku, A., Sahle, W., Tesfay, H., Bezabih, M., Aregay, A., Abera, F., *et al.* (2014). Causes of Death among Adults in Northern Ethiopia: Evidence from Verbal

Autopsy Data in Health and Demographic Surveillance System. *PLoS ONE,* **9**(**9**): e106781. doi:10.1371/journal.pone.0106781.

Mengistu, A., Enqusselasie, F., Aseffa, A., Beyene, D. (2015).Bovine Tuberculosis in Rural Ethiopia: A Comparative Cross Sectional Study on Cattle Owned by Hou seholds with and without Tuberculosis. *Mycob. Dis.* **5**:4. http://dx.doi.org/-

Mesfin, M., Tasew, W., Richard, J. (2005).The quality of tuberculosis diagnosis in districts of Tigray region of northern Ethiopia.*Health Dev.***19**:13-20.

Michel, L., Muller, B., van Helden, D. (2010). *Mycobacterium bovis* at the animalhuman interface: a problem, or not? *Vet.Microbiol.***140**: 371ï 381.

Middlebrook, G. (1954). Isoniazid-esistance and catalase activity of tubercle bacilli; a preliminary report.*American Rev. Tuberc.***69**: 471ï 2.

Mihret, A., Bekele, Y., Aytenew, M., Assefa, Y., Abebe, M., Wassie, L., *et al*. (2012). Modern lineages of *Mycobacterium tuberculosis* in Addis Ababa, Ethiopia: implications for the tuberculosis control programe. *Afri.HealthSci.* **12**(**3**):339ï 344.

Miller, B. (2000). Health sector reform: scourge or salvation for TB control in developing countries? *Int. J.Tuberc. Lung Dis.***4**(7):593-594.

Mitchison,A.(1985). The action of anti tuberculosis drugs in short course chemotherapy.*T uberculosis***66**(**3**): 219-25.

Miotto, P., Piana, F., Cirillo, M. and Migliori, B. (2008). GenoType MTBDRplus: a further step toward rapid identification of drug esistant *Mycobacterium tubercu losis*.*J. Clin.Microbiol.***46**: 393ï 394.

Moges, B., Amare, B., Asfaw, F.,Tesfaye, W., Yiruneh, M., Belyhun, Y.,*et al*. (2012). Prevalence of smear positive pulmonary tuberculosis among prisoners in Nort h West Ethiopia.*BMC Infect.Dis.***12**:352.

Moiane, I., Machado, A., Santos, N., Nhambir, A., Inlamea, O., Hattendorf, J., *et al*. (2014). Prevalence of bovine tuberculosis and risk factor assessment in cattle in rural livestock areas of Govuro District in the southeast of Mozambique.*PLoS One*, **9:** e91527.

Mokrousov, I., Otten, T., Vyshnevskiy, B. and Narvskaya, O. (2002). Detection of *em bB306* mutations in ethambutol susceptible clinical isolates of *Mycobacterium tub erculosis* from Northwestern Russia: implications for genotypic resistance testing. *J. Clin.Microbiol.***40** (**10**): 3810-3.

Mozafari, M., Farnia, P., Afraei, M., Derakhshani-Nezhad, Z., Masjedi, M. and Velayati, A. (2013). Molecular diversity of *Mycobacterium tuberculosis* strains indifferent provinces of Iran. *Iranian J Microbiol*.**5** (**4**): 366-373.

Mulisa, G., Workneh. T., Hordofa, N., Suaudi, M., Abebe, G. and Jarso,G. (2015).Multidrug-resistant Mycobacterium tuberculosis and associated risk factors in Oromia Region of Ethiopia.*Int. J Infect. Dis.* **39**: 57ï 61.

Mumpe-Mwanja, D., Verver, S., Yeka, A., Etwom, A., Waako, J., Ssengooba, W., *et al*. (2015).Prevalence and predictors of latent tuberculosis among adolescents in rural Eastern Uganda.*Afri. Health Sci.***15(3)**:851- 60.

Myneedu, V., Singhal, R., Khayyam, K., Sharma, P., Bhalla, M. and Behera, D. (2015). First and second line drug resistance among treatment naive pulmonary tuberculosis patients in a district under Revised National Tuberculosis Control Programme (RNTCP) in New Delhi. *J. Epidemiol.Glob.Health***5:** 365ï 373.

Naya, H., Romero, H., Zavala, A., Alvarez, B. and Musto, H. (2002). Aerobiosis Increases the Genomic Guanine Plus Cytosine Content (GC%) in Prokaryotes. *J. Mol. Evol.***55**(**3**): 260-264.

Ndungû,P., Kariuki, S., Ngâangâa,Z. and Revathi,G.(2012). Resistance patterns of *Mycobacterium tuberculosis* isolates from pulmonary tuberculosis patients in Nairobi. *J. Infect. Dis.* **6**(**1**):33-39.

Niemann, S., Richter, E. and Rüsch-Gerdes, S. (2000). Differentiation among Members of the *Mycobacterium tuberculosis* Complex by Molecular and Biochemical Features: Evidence for Two Pyrazinamide Susceptible Subtypes o f *M. bovis*. *J.Clin.Microbiol.***38**: 152ï 157.

Nuru, A., Mamo, G., Teshome, L., Zewdie, A., Medhin, G., Pieper, R., *et al*. (2015). Bovine tuberculosis and its risk factors among dairy cattle herds in andaround Bahir Dar City, Northwest Ethiopia. *Ethiop. Vet. J.***19**(**2**):27ï 40.

Nuru,A., Mamo,G., Zewude,A., Mulat,Y., Yitayew,G., Admasu,A.,*et al*. (2017).MedhinG, PieperR, AmeniG. Preliminary investigation of thetransmission of tuberculosis between farmersand their cattle in smallholder farmsin northwestern Ethiopia: a cross‑sectionalstudy. *BMC Res. Notes***10**:31.

Oelemann, C., Diel, R., Vatin, V., Haas, W., Rusch-Gerdes, S., Locht, C., Niemann, S., *et al*. (2007) Assessment of an optimized mycobacterial interspersed repetitive- unit-variable-number tandem-repeat typing system combined with spoligotyping for population-based molecular epidemiology studies of tuberculosis. *J. Clin.Microbiol.***45**:691-697.

OIE. (2009). Office International des Epizooties Terrestrial manual: World Health Organization for Animal Health, Paris.

Oloya, J., Opuda-Asibo, J., Kazwala, R., Demelash, B., Skjerve, E., Lund, A.,*et al*. (2008). Mycobacteria causing human cervical lymphadenitis in pastoral communities in the Karamoja region of Uganda.*Epidemiol. Infect.* **136:** 636-643.

Ombura,I., Onyango,N., Odera,S., Mutua,F. and Nyagol,J. (2016).Prevalence of Drug Resistance *Mycobacterium tuberculosis* among Patients Seen in Coast Pr ovincial General Hospital,Mombasa, Kenya.*PLoS ONE*,**11**(**10**): e0163994. doi:10.1371/.

Óreilly, M. and Daborn, J. (1995). The epidemiology of *Mycobacterium bovis* infections in animals and man: A review. *Tuber. Lung Dis* **76:** 1ï 46.

Otchere, I.,Asante Poku, A., Osei Wusu, S., Baddoo, A., Sarpong, E.,Ganiyu, A.,*et al*. (2016). Detection and characterization of drug-resistant conferring genes in *Mycobacterium tuberculosis* complex strains: A prospective study intwo distant regions of Ghana. *Tuberculosis***99**: 147e154.

Pareek, M., Evans, J., Innes, J., Smith, G., Hingley Wilson, S., Lougheed, K., *et al.* (2 012). Ethnicity and mycobacterial lineage as determinants of tuberculosis disea se phenotype. *Thorax* jnl-201824.

Parsons, L., Brosch, R., Stewart, T., Somoskövi, A., Loder, A., Bretzel,G., *et al*. (2002). Rapid and simple approach for identification of*Mycobacterum tuberculosis* complex isolates by PCR-based genomic deletion analysis. *J. Clin.Microbiol.***40**: 2339ï 2345.

Pattyn, S., Wolde, K., Hadgu, G. and van den Breen, L. (1979).Identification and drug sensitivity of tubercle bacilli from Addis Ababa, Ethiopia.*Ethiop. Med. J.***17**: 119-122.

Poulet, S. and Cole, T. (1995).Repeated DNA sequences in mycobacteria.*Arch. Microbiol.***163** (**2**); 79-86.

Radostits, O.M., Gay, C.C., Blood, D.C., Hinchelift, K.W. (2000). Disease caused by bacteria ï Mycobacterium. In: *Vet. Med.*: A Text Book of Disease of Cattle, Sheep, Pig, Goat and Horses. 9th ed. Harcourt Publisher Ltd., London, **pp**. 909-918.

Ramaswamy, V., Amin, G., Göksel, S., Stager, E., Dou, J., Sahly, H., *et al*. (2000). Molecular genetic analysis of nucleotide polymorphisms associated with ethambutol resistance in human isolates of *Mycobacteriumtuberculosis*. *Antimicrob.Agents Chemother.***44**(**2**): 326-36.

Ramirez-Busby, S., Rodwell, T., Fink, L., Catanzaro, D., Jackson, R., Pettigrove,M.,*et al*. (2017). A Multinational Analysis ofMutations and Heterogeneity inPZase, *RpsA*,and *PanD* Associated with Pyrazinamide Resistance in M/XDR *Mycobacterium tuberculosis. Sci.Reports***7**: 3790.

Raviglione, M.C., Pio, A.(2002). Evolution of WHO policies for tuberculosis control, 1948- 2001.*Lancet***359**(9308):775-780.

Regassa, A., Medhin, G. and Ameni, G. (2008).Bovine tuberculosis is more prevalent in cattle owned by farmers with active tuberculosis in central Ethiopia. *Vet. J.* **178**: 119ï 125.

Renwick, A.R., White, P.C., Bengis, R.G.(2007). Bovine tuberculosis in southern African wildlife: a multi-species hostï pathogen system. *Epidemiol. Infect.* **135:** 529ï 40.

Rinder, H. (2001). Hetro-resistance: an underrecognized confounder in diagnosis and therapy? *J. Med. Microbiol*. **50**: 1018-1020.

Rodwell, C., Moore, M., Moser, S., Brodine, K. and Strathdee, A. (2008).Tuberculosis from *Mycobacterium bovis* in binational communities, United States.*Emerg. Infect. Dis.***14**:909ï 916.

Sagonda,T., Mupfumi, L., Manzou, R.,Makamure, B.,Tshabalala, M.,Gwanzura,L.,*et al*. (2014). Prevalence of Extensively Drug Resistant Tuberculosis among Archived Multidrug Resistant Tuberculosis Isolates in Zimbabwe. *Tube rc. Res.Treat*. available at: http://dx.doi.org/10.1155/2014/349141. accessed dat e [30/11/2017].

Savine, E., Warren, M., van der Spuy, D., Beyers, N., van Helden, D., Locht, C., *et al.* (2002).Stability of variable-number tandem repeats of mycobacterial interspersed repetitiveunits from 12 loci in serial isolates of *Mycobacterium tuberculosis*. *J. Clin.Microbiol.* **40**:4561-4566.

Scorpio, A.and Zhang,Y (1996). Mutations in pncA, a gene encoding pyrazinamidase /nicot namidase, cause resistance to the antituberculous drug pyrazinamide in t ubercle bacillus. *Nat. Med*.**2**: 662ï 7.

Selgelid, M.J., Reichman, L.B. (2011). Ethical issues in tuberculosis diagnosis and treatment.*Int. J. Tuberc.Lung Dis.* **15** (Suppl 2):S9-S13.

Seyoum,B., Demissie,M., Worku,A.,Bekele,S. and Aseffa,A. (2014).Prevalence and Drug Resistance Patterns of*Mycobacterium tuberculosis* among New Smear Positive Pulmonary Tuberculosis Patients in Eastern Ethiopia. *Tuberc. R es. Treat.* available at:http://dx.doi.org/10.1155/2014/753492. accessed date [24/7/2017].

Shabbeer, A., Cowan, S., Ozcaglar, C., Rastogi, N., Vandenberg, L., Yener, B., *et al.* (2012). TB Lineage: An online tool for classification and analysis of strains of *M. tuberculosis* complex. *Infect. Genet.Evol.* **12**:789ï 797.

Shao, Y., Yang, D., Xu, W., Lu, W. , Song, H., Dai,Y., *et al.* (2011). Epidemiology of anti-tuberculosis drug resistance in a Chinese population: current situation and challenges ahead. *BMC Public Health* **11**:110 http://www.biomedcentral.com/.

Shi, W., Chen, J., Feng, J., Cui, P., Zhang, S., Weng,X., *et al*. (2014). Aspartate decarboxylase (PanD) as a new target of pyrazinamide in *Mycobacterium tuberculosis*. *Emerg.Microb. Infect*.**3**: e58; doi:10.1038/emi.2014.61.

Shirima, M., Fitzpatrick, J., Cleaveland, S., Kambarage, M., Kazwala, R., Kunda, J., *et al*. (2003). Participatory survey on zoonotic diseases affecting livestock keeping communities in Tanzania.*J. Anim. Vet. Adv.***2**: 253-258.

Shitaye, J.E., Tsegaye, W., Pavlik, I. (2007). Bovine tuberculosis infection in animal and human populations in Ethiopia: A review. *Vet. Med*. **52**(**8**): 317-332.

Sibhat, B., Asmare, K., Demissie, K., Ayelet, G., Mamo, G., Ameni, G. (2017). Bovine tuberculosis in Ethiopia: A systematic review and meta analysis. *Prev. Vet.Med.* **147**: 149-157.

Silaigwana, B., Green, E. and Ndip,R. (2012).Molecular Detection and Drug Resistance of *Mycobacteriumtuberculosis* Complex from Cattle at a Dairy Farm in theNkonkobe Region of South Africa: A Pilot Study. *Int. J. Environ. Res. Public Health* **9**: 2045-2056.

Sloot, R., Borgdorff, W., de Beer, L., van Ingen, J., Supply, P. and van Soolingen, D. (2013). Clustering of tuberculosis Cases Based on Variable-Number TandemRepeat Typing in Relation to the Population Structure of *Mycobacterium tuberculosis* in the Netherlands.*J.Clin.Microbiol.***51**(**7**):2427ï 31.

Smith, I. (2003). *M. tuberculosis* pathogenesis and molecular determinants of virulence.*Clin.Microbiol. Rev.***16**: 463-496.

Soini, H., Pan, X., Teeter, L., Musser, M. and Graviss, A. (2001). Transmission Dynamics and Molecular Characterization of *Mycobacterium tuberculosis* Isolates with Low Copy Numbers of IS6110. *J. Clin. Microbiol.* **39** (**1**):217-221.

Sola, C., Filliol, I., Gutierrez, C.,Mokrousov, I.,Vincent, V. and Rastogi, N. (2001). Spoligotype database of *Mycobacterium tuberculosis*:biogeographic distribution of shared types and epidemiologicand phylogenetic perspectives.*Emerg. Infect.Dis.* **7** (**3**):390ï 396.

Somoskovi, A., Parsons, M. and Salfinger, M. (2001).The molecular basis of resistance to isoniazid, rifampin, and pyrazinamide in *Mycobacterium tuberculosis*.*Resp. Res.* **2**:164ï 8.

Sreevatsan, S., Pan, X., Stockbauer, E., Connell, D., Kreiswirth, N., Whittam, S.,*et al.* (1997). Restricted structural gene polymorphism in the *Mycobacterium tuberculosis* complex indicates evolutionarily recent global dissemination.*Proc. Natl. Acad. Sci.* **94**:9869-9874.

Stragier, P., Ablordey, A.,Meyers, M. and Portaels, F. (2005). Genotyping *Mycobacteriumulcerans* and *Mycobacterium marinum* by using mycobacterial interspersed repetitive units. *J. Bacteriol.* **187**:1639-1647.

Srivastava, S., Garg, A., Ayyagari, A., Nyati, K., Dhole, T.and Dwivedi, S. (2006). N ucleotide Polymorphism Associated with Ethambutol Resistance in Clinical Isolates of *Mycobacterium tuberculosis. Curr.Microbiol.* **53** (**5**): 401ï 405.

Supply, P., Allix, C., Lesjean, S., Cardoso-Oelemann, M., Rusch-Gerdes, S., Willery, E., *et al*. (2006). Proposal for Standardization of Optimized Mycobacterial Inter spersed Repetitive Unit Variable Number Tandem Repeat Typing of *M. tuberc ulosis*. *J.Clin.Microbiol.* **44** (**12**): 4498-4510.

Supply, P., Lesjean, S., Savine, E., Kremer, K., van Soolingen, D. and Locht, C. (2001). Automated High-Throughput Genotyping for Study of Global Epidemiology of *Mycobacterium tuberculosis* Based on Mycobacterial Interspersed Repetitive Units. *J. Clin. Microbiol.***39** (**10**): 3563-3571.

Supply, P., Magdalena, J., Himpens, S. and Locht, C. (1997). Identification of novel intergenic repetitive units in a mycobacterial two component system operon. *M ol. Microbiol.***26** (**5**):991-1003.

Supply, P., Mazars, E., Lesjean, S., Vincent, V., Gicquel, B. and Locht, C. (2000). Va riable human minisatellite like regions in the *Mycobacterium tuberculosis* geno me.*Mol.Microbiol.***36** (**3**): 762-771.

Tadesse, M., Aragaw,D., Dimah,B., Efa, F., Abdella, K., Kebede, W.,*et al*. (2016).Drug resistance conferring mutations in *Mycobacterium tuberculosis* fro m pulmonary tuberculosis patients in Southwest Ethiopia.*Int. J.Mycobacteriol.* **5**: 185-191.

Takayama, K., Armstrong, L., Kunugi, A. and Kilburn, O.(1979).Inhibition by ethambutol of mycolic acid transfer into the cell wall of Mycobacterium smegmatis.*Antimicrob.Agents Chemother.***16**: 240ï 2.

Tamiru, F., Hailemariam, M. and Terfa, W. (2013) Preliminary study on prevalence of bovine tuberculosis in cattle owned by tuberculosis positive and negative farmers and assessment of zoonotic awareness in Ambo and Toke Kutaye districts, Ethiopia. *J. Vet. Med. Anim Health***5**: 288- 295.

Telenti, A., Philipp, J., Sreevatsan, S., Bernasconi, C., Stockbauer, K., Wieles, B., *et al*.(1997). The *emb* operon, a gene cluster of *Mycobacterium tuberculosis* involved in resistance to ethambutol. *Nat. Med.* **3**: 567ï 70.

Tessema, B., Muche, A., Bekele, A., Reissig, D., Emmrich, F. and Sack, U. (2009). Tr eatment outcome of tuberculosis patients at Gondar University Teaching Hospit al,Northwest Ethiopia. A five-year retrospective study. *BMC Public Health* **9**: 1.

Teshome, Y.(1993). Occurrence and zoonotic potential of *M. bovis* infection in Ethiopia: epidemiological, bacteriological and molecular biological aspects. DVM.*Thesis.* Giessen, Germany.

Thoen, C.O., Steele, J.H., Kaneene, J.B. (2014). Zoonotic tuberculosis: *Mycobacterium bovis* and other pathogenic mycobacteria. Oxford: John Wiley & Sons,

Tibebu, M., Mekonnen, W., Awoke, T., Gebre-Selassie, S. and Yamuah, L. (2014) A High Prevalence of Tuberculosis among Dairy Farm Workers in Addis Ababa and its Surroundings. *J. Mycobact. Dis.* **4**: 139.

Torgerson, P. and Torgerson, D. (2008). Does risk to humans justify high cost of fighting bovine TB? *Nature* **455:** 1029.

Tschopp, R., Schelling, E., Hattendorf, J., Young, D., Aseffa, A., Zinsstag, J., (2010). Repeated cross-sectional skin testing for bovine tuberculosis in cattle kept in a traditional husbandry system in Ethiopia. *Vet. Record* **167**: 250ï 256.

Tschopp, R., Hattendorf, J., Roth, F., Choudhury, A., Shaw, A., Aseffa, A., Zinsstag, J., (2012). Cost estimate of bovine tuberculosis to Ethiopia. *Curr.Top.Microbi ol.Immunol.* **365**:249ï 268.

Tsegaye, F., Defar, A., Beyene, T., Shafi, O., Klinkenberg, E. andHowe, R. (2014). Documentation and treatment outcomes of smear negative and extra pulmonary TB in Ethiopia. *Public Health Act*ion**4**: 25-S30.

Tsegaye, W., Aseffa, A., Mache, A., Mengistu, Y., Stefan, B. and Ameni, G. (2010). Conventional and Molecular Epidemiology of Bovine Tuberculosis in Dairy Farms in Addis Ababa City, the Capital of Ethiopia.The *J. Appl. Res. Vet.Med.* **8**: 143

Tsehay, R. (2002). Smallscale Milk Marketing and Processing in Ethiopia. Smallholder Dairy Production and Market Opportunity and Constraints: A South-South Workshop.

Tulu, B., Dida, N., Kassa, Y. and Taye, B. (2014). Prevalence of Smear positive pulmonary tuberculosis and its factors among tuberculosis patients. *BMC Res. Notes***7**:285.

Uplekar, M., Weil, D., Lonnroth, K., Jaramillo, E. (2015).Adopted WHO's ñGlobal strategy and targets for tuberculosis prevention, care and control after 2015. The *Lancet*. Documentation for World Health Assembly 67. http://apps.who. int/gb/ebwha/pdf_files.

Van Embden, D., Cave, D., Crawford, T., Dale, W., Eisenach, D., Gicquel, B., *et al*. (1993). Strain identification of *Mycobacterium tuberculosis* by DNA fing erprinting: recommendations for a standardized methodology. *J. Clin. Microbiol.***31**:406-409.

Van Rie, A.,Warren, M.,Beyers, N., Gie, P., Classen, N., Richardson, M., *et al.*(1999). Transmission of a multidrug resistant *Mycobacterium tuberculosis* strain resemblingñstrain Wò among noninstitutionalized, human immunodeficiency vi rus serone gative patients.*J. Infect. Dis.***180:**1608ï 1615.

Van Soolingen, D., Hoogenboezem, T., De Haas, W., Hermans, M., Koedam, A., Teppema, S., *et al*. (1997). A novel pathogenic taxon of the Mycobacterium tuberculosis complex, Canetti: characterization of an exceptional isolate from Africa. *Int. J. Syst. Bacteriol.***47**: 1236-45

Van Soolingen, D. (2001). Molecular epidemiology of tuberculosis and other mycoba cterial infections: main methodologies and achievements. *J. Intl. Med.* **249**:1-26.

Varghese, B., Hillemann, A., Wijayanti, R., Shoukri, M., Al-rabiah, F., Al-Omari, R., *et al.* (2012). New insight into the molecular characterization of isoniazid and rifampicin resistant *Mycobacterium tuberculosis* strains from Saudi Arabia. *Infect. Genet.Evol.***12**(**3**): 549-556.

Viedma, D., Lorenzo, G., Cardona, P., Rodriguez, N., Gordillo, S., Serrano, M., *et al*. (2005).Association between the Infectivity of *Mycobacteriumtuberculosis* Strains and their Efficiencyfor Extra respiratory Infection.*J. Infect. Dis.***192**:2059ï 65.

Warren, M., Van Der Spuy, D., Richardson, M., Beyers, N., Borgdorff, W., Behr, A., *et al*. (2002). Calculation of the stability of the IS6110 banding pattern in patients with persistent *Mycobacterium tuberculosis* disease.*J. Clin.Microbiol.***40** (**5**): 1705-1708.

WHO/IUATLD. (1997). Global Working Group on Antituberculosis Drug Resistance Surveillance Guidelines for surveillance of drug resistance in tuberculosis. Gen eva: World Health Organization.

WHO. (1998). Laboratory Services in Tuberculosis Cont*rol: Culture. Part III*, World Health Organization, Geneva, Switzerland.

WHO.(2011).Tuberculosis prevalence surveys:a handbookWorld Health Organization Geneva, Switzerland.

WHO (2014). End TB Strategy.WorldHealthOrganization.Available at [http://www.who int/tb/post2015_strategy/en/].

WHO. (2015). The End TB strategy. World Health Assembly. http://www.who.int/tb/strateg accessed date [14/11/2017].

WHO. (2017). Global tuberculosis report. World Health Organization. Geneva, Swizerland, available at: http://www.who.int/tb/publications/global_report/ accessed date [26/10/2017].

WHO (2018). Global Tuberculosis Report. World Health Organization. http://www.who.int/tb/strateg accessed date [15/01/2019].

Wolde, K., Lemma, E. and Abdi, A. (1986). Primary resistance to the major anti-tuberculosis drugs in Ethiopia.*Ethiop.Med. J.* **24**(**1**):15-8.

Wondimeneh, Y., Muluye,D. and Belyhun,Y. (2012).Prevalence of Pulmonary tuberculosis andimmunological profile of HIV co-infected patientsin Northwest Ethiopia. *BMC Res. Notes***5**:331.

Wood,R., Liang,H., Hulin, W., Middelkoopa,K., Onid,T. and Rangaka,M. (2010). Changing prevalence of TB infection with increasing age in high TB burden townships in South Africa.*Int. J. Tuberc. Lung Dis.* **14(4)**: 406ï 412.

Young, D., Gideon, H. and Wilkinson, R. (2009). Eliminating latent tuberculosis.*Trends Microbiol.* **17**(**5**): 183-188.

Zelalem, Y. and Faye, B. (2006). Handling and microbial load of cowôs milk and irgo-fermented milk collected from different shops and producers in central highlands of Ethiopia. *Ethiop. J. Anim. Prod.***6** (**2**): 67-82.

Zenebe, Y., Anagaw, B., Tesfay, W., Debebe, T. and Gelaw, B. (2013). Smear positive extra pulmonary tuberculosis disease at University of Gondar Hospital, Northwest Ethiopia. *BMC Res. Notes***6**:21.

Zignol, M., Dean, A., Falzon, D., van Gemert, W., Wright, A., van Deun, A.,*et al*. (2016). Twenty Years of Global Surveillance of Antituberculosis DrugResistance. *N. Engl. J. Med.***375**:11.

Zhang, Y. (2005). The magic bullets and tuberculosis drug targets.*Annu. Rev. Pharmacol. Toxicol.***45**: 529ï 64.

Zhang, Y. and Yew, W. (2009). Mechanisms of drug resistance in *Mycobacterium tuberculosis. Int. J. Tuberc. Lung Dis.***13 (11):** 1320-1330.

Zhao, L., Sun, Q., Liu, H., Wu, X., Xiao, T., Zhao, X., *et al*. (2015). Analysis of *embCAB* mutations associated with ethambutol resistance in multidrug-resistant*Mycobacterium tuberculosis* isolates from China. *Antimicrob. Agents Chemother.***59**: 2045ï 2050.

APPENDICES

Appendix I. Informed Consent form for individuals who attend health centers /hospital in South Gondar Zone of the Amhara Region, and who are inviting to participate in research on tuberculosis. The title of the research project is ñ**Tuberculosis in Farmers and Their Cattle in Smallholder Farmi -ng System in South Gondar Zone of northwest Ethiopia**: Epidemiology an d Drug Sensitivity Profiles

This Informed Consent Form has two parts:

- **Information Sheet (to share information about the research with the attendants)**
- **Certificate of Consent (for signatures if the attendants agree to take part in the research)**

The attendants will be given a copy of the full Informed Consent Form

PART I: Information Sheet

Introduction

I am Mr. Amir Alelign, a PhD student at Addis Ababa University. We are doing research on cattle and human tuberculosis disease, which is very common in this country. I am going to give you information and invite you to be part of this research. You do not have to decide today whether or not you will participate in the research. Before you decide, you can talk to anyone you feel comfortable with about the research.

There may be some words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later, you can ask them of me, the study doctor or the staff.)

Purpose of the research

Globally, tuberculosis (TB) causes millions of deaths per year. Ethiopia ranks seventh among the worldǿs 22 countries with high tuberculosis burden. *Mycobacterium* *tuberculosis(M. tuberculosis)* is the most common cause of human TB, but an unknown proportion of cases are due to *M. bovis*. Although cattle are considered to be the main hosts of *M. bovis*, isolations have been made from many other livestock and wildlife species and transmission to humans constitutes a public health problem. TB caused by *M. bovis* is clinically indistinguishable from TB caused by *M. tuberculosis* and can only be differentiated by laboratory methods. BTB became rare in human and cattle in developed countries as the result of milk pasteurization and test and slaughter policy.

Tuberculosis is one of the most common and dangerous diseases in this region. Bovine tuberculosis is known widely distributed in north western Ethiopia, however, the situation is not well known among farmers. Particularly, in South Gondar Zone, no enough recorded data on the transmission of the disease from cattle to humans.

Considering the above facts, it is planned to undertake a research on cattle and human tuberculosis in the next two years among farmers in south Gondar zone of the Amhara region. The research will be undertaken with coordination involving many profession al personnel mainly cattle and human health experts as well as researchers will participate.

The objectives of the study are:

a) To determine the magnitude in the distribution of tuberculosis in South Gondar Zone

b) To determine the zoonotic transmission of bovine tuberculosis in the study area.

c) To investigate the possible distribution *M. bovis* strains in the study area

d) To investigate the association of different risk factors with BTB prevalence and specific strain of *M. bovis.*

With this respect, a research on cattle and human tuberculosis will be undertaken at the woreda health centers and fields in south Gondar zone.

Type of Research Intervention

This research will involve collection of a sputum sample (during the first health center visit) or a biopsy at swollen lymph nodes from TB suspected individuals, **only sharing the samples from the routine TB diagnosis at health centers (Samples**

will not be taken only for the purpose of the research), an interview as well as a single injection at two sites in the neck region of your cattle at the field.

Participant selection

We are inviting all individuals above five years of age with tuberculosis who attend health centers in South Gondar Zone to participate in the research on cattle and human tuberculosis.

Voluntary Participation

Your participation in this research is entirely voluntary. It is your choice whether to participate or not. Whether you choose to participate or not, all the services you receive at this clinic will continue and nothing will change. If you choose not to participate in this research project, you will be offered the treatment that is routinely offered in this clinic/hospital for TB, and we will tell you more about it later. You may change your mind later and stop participating even if you agreed earlier.

Procedures and Protocol

You will receive the treatment of your condition according to national guidelines. This means that you will be given anti-TB drugs and consultations free of charge from the health center.

Description of the Process

During the research you make only one visit to the health center and we will make two visits to your farm land where your cattle are found.

- During your visit to the health center/hospital in seeking TB treatment, a small amount of sputum, equal to about a teaspoon, will be collected from your mouth with a small container. This sputum will be tested for the presence of bacteria that cause human or cattle tuberculosis. We will also ask you a few questions about your habit of consuming raw milk and diary products, raw meat and your association with cattle at your home or farm, in addition, we will also ask you about your general awareness about human and cattle TB.

- In the presence of swellings as TB indications and to confirm the cause of your swelling, a small sample of your skin will be taken. The guidelines say that the sample must be taken using a local anesthesia which means that we will give you an injection close to the area where we will take the sample from. This will make the area numb so that you will not feel any pain when we take the sample.
- After one month, we will visit your farm land to test your cattle for the presence of cattle TB. (This will involve a single injection at two small shaved sites at the neck region and measuring the swelling after three days).
- Lastly, based on your willingness and sale agreement, those cattle found to be strongly TB-positive will be sacrificed for further investigation.

Risks

By participating in this research it is possible that you will feel some discomfort and may lose some time in coming to the health center otherwise there will not be given any new medicine or treatment that might harm your health. The fine needle aspirations (FNA) will be carried out by experienced pathologists at hospital level as part of the routine diagnosis using appropriate aseptic (safety) measures. Commonly, you may feel a little sore for a couple of days after the test. You may develop a bruise at the site where the needle was inserted. Otherwise, complications are uncommon. But if it happens, you will get appropriate treatment at the hospital.

Benefits

There may not be any benefit for you but your participation is likely to help us find the answer to the research question. There may not be any benefit to the society at this stage of the research, but future generations are likely to benefit.

Reimbursements

We will give you 15 birr to pay for your travel to the health center and we will give you 35birr for lost work time. For those who are willing to scarify their TB positive cattle an agreed compensation will be paid. All cattle participated in the skin test will be treated with antihelmenthic drug as incentives. Otherwise, you will not be given any other money or gifts to take part in this research.

Confidentiality

The information that we collect from this research project will be kept confidential. Information about you that will be collected during the research will be put away and no-one but the researchers will be able to see it. Any information about you will have a number on it instead of your name. Only the researchers will know what your number is and we will lock that information up with a lock and key. It will not be shared with or given toanyone except Addis Ababa University and the health officials at the health center. The biological samples obtained during this research procedure will be used only for this research, and will be destroyed after two years, when the research is completed.

Sharing the Results

The knowledge that we get from doing this research will be shared with you through community meetings before it is made widely available to the public. Confidential information will not be shared. There will be small meetings in the community and these will be announced. After these meetings, we will publish the results in order that other interested people may learn from our research.

Who to Contact

If you have any questions you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact any of the following:

Prof. Gobena Ameni: *Addis Ababa University, Aklilu Lemma Institute of Pthobiology. Office tel.: +251-112 76309, Mobile: +251-911 413073, University email ID: gobena.ameni@aau.edu.et*

Amir Alelign: AddisAbaba University, College of Life Science, Department of Microbial, Cellular and Molecular Biology. Office tel: +251-8959216, Mobile: 0911380705. Email ID: negaalelign@yahoo.com

This proposal has been reviewed and approved by Addis Ababa University, College of Natural Science IRB, which is a committee whose task it is to make sure that research participants are protected from harm.

PART II: Certificate of Consent

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Name of Participant__________________

Signature of Participant ___________________

Date ___________________________

Day/month/year

If illiterate

A literate witness who is selected by the participant will sign.

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Name of witness_____________________ AND Thumb print of participant

Signature of witness ______________________

Date ________________________

Day/month/year

Statement by the researcher/person giving consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:

1. Sputum samples or a biopsy at swellings will be collected

2. Skin test on cattle will be done at the farm land of the participants

3. TB positive cattle will be sacrificed in agreement with the owners and samples will be taken for further study.

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Name of Researcher/person taking the consent________________________

Signature of Researcher /person taking the consent__________________________

Date ___________________________

Day/month/year

Appendix II. Checklist of symptoms in TB suspects in the community

Questionnaire No.________ Name of interviewer__________

Date___________________

1. Socio-demographic variables

1.1 Name of suspect ____________________ 1.2. Age________1.3. Sex _____________

1.4. District __________ Kebele _________

1.5. Marital status Single___________ Married__________ Divorced________________

Widowed________ other (specify) ____________________________

1.6. Educational status: Illiterate ____ Grade level ____ other (specify) ______________

2. Tuberculosis symptoms and history

2.1. Did you experience cough for two or more weeks? Yes __________ No ________

If yes, for how many weeks _________

2.2. Is the cough productive of sputum? Yes __________ No ________

If yes, does it contain blood? Yes __________ No ________

2.3. Did you have fever and night sweats? Yes __________ No ________

If yes, for how many weeks _________

2.4. Did you have loss of appetite? Yes __________ No ________

If yes, for how many weeks _________

2.5. Did you loss weight? Yes __________ No ________

2.6. Did you have chest pain? Yes __________ No ________

If yes, for how many weeks _________

2.7. Did you have shortness of breath? Yes _________ No ________

If yes, for how many weeks _________

2.8. Did you have history of tuberculosis treatment? Yes __________ No ________

2.9. Did you have closer contact with known TB patient? Yes ________No ________

Appendix III. Laboratory Register

Laboratory register for AFB **Month(s):__________ Year:_____________**

Patient No.	Health unit	Date	Lab. Serial No.	Name and address of patient	Age	Sex	Name and address of contact person	Patient category		TB type		Results		Remark
								New	Retreatment	PTB	EPTB	1	2	

Appendix IV.Populationperception and exposure for the possible risk factors of zoonotic transmission of TB to cattle owners/attendants.

No.	Possible Risk factors		Yes		No	Remarks
1.	Presence of TB patient in your home or family					
2.	Milk consumption habit	Raw milk				
		Boiled milk				
		Both raw and boiled milk				
		Do not drink				
		Sour milk (yoghurt)				
3.	Meat consumption habit	Cooked meat				
		Raw meat				
		Both raw and cooked meat				
4	Condition of cattle Keeping	Separate places				
		Close proximity to owner's house				
		Sharing shelter				
5	Use of cow dung	Plastering wall and floor				
		Source of energy for cooking				
		Not use at all				
6.	Knowledge of BTB					
		knew or have not heard about TB of cattle				
		recognize the symptoms				
		identify the post-mortem tissue lesions				
		BTB transmission between the cattle population				
		possible transmission of the disease from sick cattle to humans -inhalation -ingestion of contaminated milk and meat - any unique practice				

Appendix V. General characteristics of cattle to undergo tuberculin skin test

Study site (District)______________________

Name of Cattle owner (attendant)______________________ Date_______________

Cattle code	Categories													
	Age	Sex		Breed		Body condition			Pregnancy status	Tuberculin skin test measurement (reading)				
		M	F	Indigenous	Hybrid	Poor	Medium	Good		A_1 B_1	A_2	B_2 B-	A	△ △
Coo1														
C002														
C003														
C004														
C005														
C006														
C007														
C008														
C009														
C010														
C011														
C012														
C013														
C014														
C015														
C016														
C017														
C018														
C019														
C020														

A1: Avian reading day 1; A2: Avian reading day 2; B1: bovine reading day 1; B2: Bovine reading day 2. △B: Change in reading of bovine (B 2-B1); △A: change in reading of avian PPD test (A2-A1).

Addis Ababa University

School of Graduate Studies

Declaration

This is to certify that the thesis prepared by Amir Alelign, entitled: **Tuberculosis in Farmers and Their Cattle in Smallholder Farming System in South Gondar Zone of northwest Ethiopia: Epidemiology and Drug Sensitivity Profiles** and submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy (Biomedical Sciences) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed.