Tuberculosis Control in Arsi in Ethiopia:

Programme Performance and Disease Burden

:

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Lists of publications

This thesis is based on four original papers, which are referred to in the text from Roman numerals I to IV:

Paper I: Hamusse S., Lindtjorn B.; Demissie M. Trends in TB Case Notification over Fifteen Years: The case notification of 25 Districts of the Arsi Zone of Oromia Regional State, Centeral Ethiopia. BMC Public Health 2014, 14:304.

Paper II: Hamusse S., Demissie M., Teshome D., Lindtjørn B. (2014). Fifteen-year trend in treatment outcomes among patients with pulmonary smear-positive tuberculosis and its determinants in the Arsi Zone, Central Ethiopia. Glob Health Action 2014; 7: 25382.

Paper III: Hamusse S., Demissie M., Teshome D., Hassen M., Lindtjørn B. Prevalence and Incidence of Smear-Positive Pulmonary Tuberculosis in the Hitossa District of the Arsi Zone, Oromia Regional State of Central Ethiopia. 2016; re-submitted after revised for minor revision to BMC infectious disease journal for publication.

Paper IV: Hamusse S., Demissie M., Teshome D., Hussen M., Lindtjørn B. Primary and secondary anti-tuberculosis drug resistance in the Hitossa District of the Arsi Zone, Oromia Regional State, Central Ethiopia. BMC Public Health 2016; 16:593.

List of abbreviations

AFB	Acid Fast Bacilli
AID	Acquired Immunodeficiency Syndrome
ART	Annual risk of tuberculosis infection
BCG	Bacille Calmette Guerin
CDR	Case Detection Rate
CNR	Case Notification Rate
CSA	Central Statistics Agency
DM	Diabetes mellitus
DOTS	Directly Observed Treatment, Short Course
DST	Drug susceptibility Test
EPTB	Extra-Pulmonary Tuberculosis
EMB	Ethambutol.
FMOH	Federal Ministry of health of Ethiopia
GDP	Gross Domestic Product
HBCs	High-burden countries
HEP	Health Extension Program
HEWs	Health Extension Workers
HIV	Human Immunodeficiency Virus
HSTP	Health Sector Transformation Plan
IGRA	Interferon-gamma release assays
INH	Isoniazid
IUATLD	International Union against Tuberculosis and Lung Disease
LED	Fluorescent light-emitting diode
LPA	Line probe assay
LTBI	Latent Tuberculosis Infection
MDGs	Millennium Development Goals
MDR	TB Multidrug Resistant Tuberculosis
MMR	Miniature Mass Radiographs
MTB	Mycobacterium Tuberculosis

NTLCP	National Tuberculosis and Leprosy Control Programme
PCR	Polymerase chain reaction
PTB	Pulmonary Tuberculosis
SSPTB	Symptoms suggestive of TB
TSR:	Treatment Success Rate
RIF	Rifampicin
SDGs	Sustainable Development Goals
STM	Streptomycin
TST	Tuberculin skin test
WHO	World Health Organization
XDR-TB	Extensively Drug-Resistant TB
ZN	Ziehl-Neelsen

Summary

Tuberculosis (TB) remains a high-priority communicable disease that causes an enormous burden of morbidity and mortality, and infects one-third of the world's population. It is the second leading cause of death among infectious diseases worldwide, with more than one-fourth of all preventable adult deaths in developing countries due to TB. The disease disproportionately affects people in resource-poor settings, particularly those in Asia and Africa. In addition, more than 80% of TB cases and 78% of deaths occur in developing countries.

The primary causes of the TB epidemic in developing countries are poor socio-economic conditions, an increase in human immuno-deficiency virus and an increase in anti-TB drug resistance, especially the MDR-TB strain. The weak and ineffective national TB control programmes, the poor implementation of infection prevention measures, the poor quality and accessibility to anti-TB drugs, the irrational use of anti-TB treatment regimens and the poor patient adherence are the underlying causes for the emergence of drug- resistant strain including MDR-TB. The emergence of this strain therefore poses another challenge to TB control efforts.

TB control aims at detecting infectious TB cases as early as possible, and puts them on standardized anti-TB treatment in order to successfully treat and break the chain of transmission and to avert the emergency of multi-drug resistance. The effectiveness of the TB control strategy mainly depends on the timely diagnosis and treatment of smear-positive pulmonary tuberculosis. The cure of smear-positive pulmonary TB patients is considered to be an important intervention mechanism for the primary prevention and emergency of MDR-TB. As a result, a rapid identification of smear-positive pulmonary TB cases and their effective treatment using combined anti-TB drugs is the cornerstone of the global TB control programme.

In 1993, the World Health Organization (WHO) confirmed TB as a global public health emergency and recommended the Directly Observed Treatment, Short Course (DOTS) as a standard strategy to control the disease. In 1994, the World Health Organization designed a Framework for Effective Tuberculosis Control, which clearly designated the core elements of the DOTS strategy. DOTS aims at detecting 70% of infectious TB cases and successfully treating

85% of them to interrupt the transmission, reduce mortality and prevent the emergence of drug resistance.

Ethiopia is among the 22 high-TB-burden countries and the 27 high-MDR-TB-burden worldwide. TB is the leading cause of hospital admission and second leading cause of death in the country. The WHO recommended the DOTS strategy, which was piloted in 1992 and nationally launched in 1995, in a few health facilities with a subsequent expansion to all public health institutions. As a result, in 2015 all public and 14% of private health institutions were covered. The overall aim of this thesis is to assess the trends in TB control performance, and to estimate the burden of the disease at the community level to help achieve a better understanding of the gap in improving the TB control programme in Ethiopia.

The thesis investigates TB control performance, and estimates the disease burden at the community level. The studies focus on assessing trends in TB case notification and treatment outcomes. In addition, we estimate the prevalence, incidence of bacteriologically confirmed TB cases, as well as the burden of primary and secondary drug resistance TB at the community level. The findings of the studies could also be used to explore area-specific strategies help to improve TB control programmes in Ethiopia.

The studies were conducted in Arsi in central Ethiopia, and used cross-sectional and prospective cohort study designs. The studies were conducted in predominantly rural communities and at public health institutions. Most of the papers focus on smear-positive TB, the most infectious form of TB.

The study findings show that the trend in PTB+ case notification increased in parallel with the expansion of DOTS population coverage from 18% to 70% over 15 years. The PTB+ case notification increased from 7 to 63 per 100,000 population in 15 years, with an overall increase of 89%. The TB case detection rate (CDR), estimated by the proportion of PTB+ cases notified from the total annual expected PTB+ incidence of the zone, went up from 6.4% to 58.7% over the study period. The overall 15-year average PTB+ case CDR of the zone was 37.7%, which was far below the 70% global target. Moreover, the PTB+ case notification varied across the 25

districts of the zone. The rural residence and population ratio to DOTS sites and age of the patients were associated with a low TB case notification.

Between 1997 and 2011, the treatment success rates for smear-positive TB rose from 61% to 91%, with a corresponding decline in treatment failure and default rates. The 15-year average cure rate was 67%, which was lower than the global target of an 85% of cure rate. However, treatment outcomes varied across the 25 districts of the zone. The treatment success rate was also found to be associated with the age of the patient, the patient category and TB/HIV co-infection.

Trends in case notification and treatment outcomes are used as proxy indicators to evaluate the TB programme performance. However, to obtain a better understanding of the impact of the TB control programme, we need both baseline and follow-up data on the disease prevalence, incidence and drug resistance burden at the community level. Considering the shortage of resources, we used a less expensive method to estimate the prevalence and incidence of PTB+ and primary and secondary drug resistance, using symptom inquiry followed by sputum microscopy for AFB, culture and a drug-susceptibility test.

The results show that there is a high incidence of PTB+ cases. For every case PTB+ on anti-TB treatment, there was an almost equal number (0.96) of undiagnosed BCTB cases in the community. Furthermore, we identified more men undergoing treatment before the survey, whereas more women were detected during the active TB case finding. The history of TB contact was found to increase the risk of developing active TB, thus suggesting the targeting of contact-tracing among household members diagnosed with PTB+ to help capture the undetected infectious TB cases in the community. The estimation of TB prevalence and incidence based on symptom inquiry and sputum microscopy is a less expensive and simple technique. This method might help to generate information on the magnitude of TB in resource-constrained settings.

We also found that there is a high prevalence of primary and secondary resistance to any one or more first-line anti-TB drugs and primary and secondary MDR-TB in the study area. The highest prevalence of secondary drug resistance was identified among previously treated TB cases compared to primary resistance among new TB cases. This is primarily due to the poor treatment

outcomes among previously treated cases caused by lost follow-up and irregularity of drug intake.

The overall 15-year average PTB+ case CDR was 38%, while the cure rate was 67%. So, after 15 years of the DOTS programme, the high proportion of undetected infectious TB cases in the community, combined with increasing primary and secondary drug resistance TB, we conclude that there has been a sub-optimal DOTS performance. Hence, this thesis underscores the need to improve DOTS performance through devising alternative strategies in TB control programmes in Ethiopia.

Introduction

Tuberculosis: Historical aspects

Tuberculosis (TB) has been known to mankind, and claimed an unlimited amount of lives since ancient times. It was referred to by several terms including ***consumption,*** which implied a severe weight loss, and a white plague due to extreme pallor and phthisis [1-3]. In the Western world, the communicability and clinical features of TB were known prior to 1000 BC. However, current advances in molecular technology identified the existence of TB in the relics and bone samples from some 15,000 to 20,000 years ago [4-6]. Moreover, archaeologists detected spinal TB, known as Pott's disease, from Egyptian mummies. Evidence of the tuberculosis of lymph nodes, named scrofula, was also identified during the Middle Ages [4, 6, 7].

As a causative agent for tuberculosis, *mycobacterium tuberculosis*, was discovered by Koch in 1882. He showed the unique protein coat of *mycobacterium tuberculosis,* which made it difficult to visualize. An acid-fast staining technique called the Ziehl Neelsen stain was then developed for the identification of mycobacterium in specimens [1]. Since that time, sputum microscopy, which uses acid-fast staining, has been utilized as an important diagnostic tool. Moreover, in 1895, 13 years after the discovery of *Mycobacterium tuberculosis*, Wilhelm Roentgen used X-ray technology for the first time, which later played a pivotal role in the diagnosis of pulmonary tuberculosis [1,2].

Thirty-nine years after this historical milestone, in 1921, French scientists Albert Calmette and Camille Guerin developed Bacille Calmette-Guerin (BCG) vaccine against TB. Subsequently, research conducted during the first half of the 20th century led, in 1944, to the discovery of two chemotherapeutic agents, streptomycin and para-amino alicyclic acid (PAS) [1, 8]. This paved the way in the scientific field for the succeeding discovery of isoniazid in 1951, and in the late 1960s of Rifampicin as an effective anti-TB drug.

Tuberculosis increased dramatically during the 17th and 18th centuries in Europe and North America. The death rate from the disease reached its peak in 1800, accounting for one in every four deaths in England and New York City [1, 9]. The high morbidity and mortality rates that prevailed in Europe and North America during those days were linked to overcrowding, low

socio-economic conditions that resulted from poor nutrition, a lack of hygiene and sanitation, and a lack of medical care [1, 10, 11].

However, following the discovery of M. *tuberculosis* in 1882 by Robert Koch and X-rays in 1895 by Wilhelm Konrad Roentgen, death rates from tuberculosis began to decline. The discoveries made the diagnosis of TB simpler through the use of radiography to demonstrate abnormalities in a patient's chest and the identification of tubercle bacilli in sputum. These tools subsequently improved the management of the disease [1]. During the second half of the 20th century, the advancement of science in the areas related to the diagnosis, and the management of TB and the socio-economic improvement in high-income countries, played a pivotal role in the reduction of TB incidence and deaths due to the disease [12]. Although a fall in TB incidence was primarily noted in high-income countries, the disease continued to be the leading cause of sickness and deaths in poor-resource countries [13].

The steadily declining trend in TB incidence between 1953 and 1985 in the United States was followed, during the early 1970s, by a substantial budget cut from the global TB research and drug development priority list [1]. However, in 1986, following the emergence and spread of the HIV infection, an increase of TB incidence and drug resistance was recorded in New York City, with the epidemic reaching its peak in 1992 [1, 9, 11]. This was the historical time at which TB was recognized as a global threat, and later declared as a global emergency. Since 1994, the Directly Observed Treatment, Short Course (DOTS) has been recommended as a standard strategy to control the disease [14].

Tuberculosis transmission and its course of infection

Tuberculosis is a bacterial disease caused by *M. tuberculosis,* which is spread by airborne droplet nuclei consisting of tiny particles 1–5μm in diameter that contain *M. tuberculosis*. The bacilli are expelled and suspended in the air as airborne droplet nuclei when people with pulmonary tuberculosis cough, sneeze, talk or sing [15-17]. For instance, an active TB case could discharge approximately 3,000 droplets of nuclei during an episode of coughing or talking for five minutes [18]. *Mycobacterium tuberculosis* is rod-shaped, non-spore forming, and is neither gram-positive nor gram-negative aerobic bacteria. Because of its thick cell wall, the bacterium does not decolourize after staining with acid, and is therefore known as acid-fast bacilli (AFB) [19].

Tuberculosis primarily affects the lungs, and can then spread through the blood stream, the lymphatic system or through airways to other parts of the body and organs [20]. Extra-pulmonary TB accounts for nearly 20% of the disease among HIV sero-negative individuals, but is more common among HIV sero-positive people [21]. Patients who are sputum smear-positive for *M. tuberculosis* pulmonary TB are the principal sources of infection [22-24]. However, the clinical presentation of TB varies and depends on the duration and site of the disease. Whatever the case, the most classic symptoms of pulmonary PTB include coughing for three or more weeks, haemoptysis in advanced cases, weight loss and a low-grade fever.

Nevertheless, PTB can also occur without cough, and it may be asymptomatic [25-27]. EPTB can exhibit site-specific symptoms, including lymph node swelling in lymphatic disease, neck stiffness/neurologic symptoms in meningeal TB, lower back pain in spinal TB, bone pain or joint swelling in osteomyelitis and infertility if it involves the genitourinary system [25, 26]. Understanding the clinical presentation and symptoms of TB is important for the community in seeking medical advice, and to inform service providers so that they can identify presumptive TB cases and treat them in time.

One of the main immuno-pathological characteristics of active pulmonary TB is the formation of cavities in the lung. These cavities tend to harbour a large amount of bacteria; thus, TB patients with cavitary lesions have a high mycobacterial load, and are more infectious than non-cavitary cases [28-30]. Even so, extra-pulmonary TB (EPTB) is generally non-infectious, and thus has a lower public health priority [25, 26].

The risk of infection with tubercle bacilli primarily depends on the incidence of infectious TB cases in the community, the period of their infectiousness and the number and nature of contacts between an infectious case and a susceptible individual per unit time of infectiousness [31, 32]. The risk of being infected from a single contact with an infectious case is determined by the extent of the close contact, the length of exposure and the amount of TB bacilli in the sputum of infectious TB cases [33]. A single untreated infectious case can infect approximately 5-10 individuals every year, and can generate about 20 patients for an average two-year period [31].

Primary TB infection occurs when a newly infected person is exposed to tubercle bacilli. Following the lodging of bacilli into the terminal alveoli of the lungs, the bacilli begin to multiply and form a primary complex lesion known as a Ghon focus [34, 35]. The inhalation of droplet nuclei initiates an immune response resulting in any of the following three clinical outcomes: a complete clearance of the bacteria and self-cure [36], latent TB infection (LTBI) or progression to primary active TB [37].

Following primary TB infection, the host immune response may prevent the multiplication of the bacilli; nonetheless, in most cases the bacilli are not fully eradicated, thereby eventually resulting in a latent TB infection [38]. Individuals with this type of infection are apparently healthy, with no evidence of infection unless they are identified using a tuberculin skin test (TST) or interferon-gamma release assays (IGRAs) [39].

Approximately two to three billion of the world's population are estimated to be infected with *M. tuberculosis* [40]. Individuals infected with tubercle bacilli can progress to being an active TB patient at any time, depending on their age, duration of infection and their immunity. Those with a latent TB infection have an approximately 10-20% risk of progressing to active TB during their lifetime [32, 41-43], with the risk usually high during the first two years following infection [44]. It increases from 1.5% during the first year to 5-10% within the first five years, and then declines to 5% after that [45]. The overall risk of latent TB becoming active may increase following the impairment of the host immune system, such as with an HIV infection, malnutrition, immuno-suppressive treatments, old age, tobacco use and alcoholism [46-48].

The risk of progression to active TB is raised by HIV co-infection [49, 50]. The annual risk of developing active TB among people co-infected with TB/HIV is as high as 10%, and may even be higher with severe immuno-suppression [51-53]. In the absence of treatment, 50% of pulmonary TB patients usually die within five years, 25% self-cure, and 25% continue to be chronic infectious TB cases [54].

Moreover, under an inappropriately handled tuberculosis control programme, approximately 30% of people with smear-positive pulmonary tuberculosis cases die [55]. Nevertheless, under the WHO's Stop TB Strategy, TB case fatality rates all over the world are less than 5% [56]. In

general, properly treating TB cases, using appropriate, good quality and adequate chemotherapy, not only prevent deaths resulting from the disease, but also cures the case and prevents it from developing into a chronic infectious form that could be a source of infection to others [57], subsequently reducing the risk of drug resistance [58].

However, much higher death rates were reported from patients treated for HIV-associated TB [59-69]. The overall case fatality rate of HIV-infected TB cases was reported to be 40% across different countries [70]. In Sub-Saharan Africa, in the absence of prophylactic treatment for opportunistic infections and anti-retroviral treatments, approximately 30% of smear-positive pulmonary TB cases co-infected with HIV died within 12 months of starting treatment, and about 25% of those who completed treatment died during the following 12 months [71].

Determinants of tuberculosis infection and disease

The development of TB in a person is a two-stage process, in which a susceptible individual is exposed to an infectious TB case and becomes infected, and may later develop active TB. The progression of TB infection to active TB is determined by different conditions that may change the balance between the host immune defence mechanism and the tuberculin bacilli [72].

The risk of a susceptible individual to be infected with TB bacillus depends on the infectivity of the source infection, the frequency and time of exposure to the infectious case and the degree of susceptibility of the exposed individual. The infectivity of infectious TB case increases with the frequency of coughing, sneezing, time of talking and number bacilli in the sputum [73]. Individuals infected for the first time or re-infected can be identified by either a Mantoux tuberculin skin test (TST) or interferon-gamma release assays (IGRAs). The proportion of population who are newly infected or re-infected over one year can be measured by the annual risk of tuberculosis infection (ARTI) at the population level [74]. A relatively higher ARTI, ranging between 1% and 2%, is observed among poor countries when compared with developed countries having an ARTI that ranges between 0.1% and 1% [75]. The high range among developing countries is an indication of a disproportionally high rate of TB transmission in poor resource settings.

After infection, progression to active TB take place at different time intervals either due to the reactivation of latent TB or an exogenous re-infection [31]. The reactivation of latent TB or re-infection could vary with a difference in TB epidemiology across different settings [42]. New infections and re-infections among adult population are mainly observed among high TB-burden countries, while the reactivation of latent TB is seen in countries with a low burden of TB [31, 42, 76]. This dichotomy in TB burden between the developing and developed countries could be due to a variance in socio-economic and demographic factors and HIV burden among these countries [77, 78]. In general, factors determining TB infection and its progression to an active disease are presented as follows.

Age

Differences in TB infection and disease burden across various age groups have been reported from different parts of the world. The risk of infection increases from early infancy to early adult life, possibly due to the increasing number of social interactions and frequency of contacts [79]. The incidence of TB rises from early infancy to pre-adolescence, but falls as the time from of infection increases. Moreover, young children often developed disseminated TB such as TB meningitis and extra-pulmonary TB, which affected organs other than the lungs as a result of immature cellular immunity to localized TB bacilli [45].

Tuberculosis is predominantly reported to be a disease among the adult population in the productive age group from 15 to 49 years [80]. Moreover, the risk of the disease increases after 60 years of age due to an increased underlying medical condition that might elevate the risk for the reactivation of latent TB at an older age [81]. In a population with a high TB transmission, a greater incidence of TB is usually observed among children due to an ongoing infection. On the contrary, in a population with a low TB transmission, the burden shifts to older adults due to the reactivation of latent TB at a later age [82, 83].

Gender

Globally, a high number of TB cases is reported among men compared to women [84, 85]. For instance, from the total of 8.6 million estimated TB incidents among the adult population in 2014, 5.4 million were males and 3.2 million females [86]. This indicates that for every one case of TB incidence among females, there were about 1.7 incidences of TB cases among males. The difference in TB incidence among males and females might be explained by the biological difference between men and women. However, the progression of TB infection to disease is high among women in the reproductive age group compared to men. The socio-cultural factors, including an inability to make decisions on resources, stigma and poor health-seeking behaviour may hinder a women's ability to utilize the existing health services [87-89]. Some study reports also indicate that hormonal factors might play a role in the risk of tuberculosis infection and its progression to active disease [90, 91].

The dichotomy of HIV infection among women compared to men could make women more susceptible to developing active TB. Tuberculosis is the second leading cause of death among the infectious diseases next to HIV in young women in developing countries [92, 93]. Hence, studies that could help to understand the interaction between the biological, health system and socio-cultural determinants of gender-based variances are needed to be undertaken.

Tuberculosis and HIV co-infection

HIV is known to be the most powerful determinant in increasing the risk of TB infection and its progression to active disease [9, 94, 95]. Approximately 80% of the total of estimated HIV-associated TB is found in the countries of Sub-Saharan Africa [96], with TB among the leading causes of morbidity and mortality in HIV-infected individuals already having TB [97].

The interaction of the two deadly diseases resulted in the loss of human assets with a subsequent impact on the socio-economic growth and overall development of the countries. In 2014, of the total 1.2 million of TB/HIV co-infected cases, 74% were in Africa and 32.5% (390,000) of them died [86]. This suggests that TB/HIV co-infection greatly increases the risk of mortality if both infections are not properly addressed [98]. However, the early detection of TB cases and the

prompt initiation of treatment could decrease morbidity and mortality resulting from TB disease among HIV-infected individuals [99].

Tuberculosis infections among immune competent individuals remain asymptomatic and become latent infections. Still, there is a high risk of for the progression of a primary infection to active TB among HIV infected individuals. Likewise, the risk of a latent TB progression to an active one among HIV-infected individuals is roughly 20-30 times more likely compared to non-HIV infected ones [100]. Moreover, the treatment of active TB among HIV co-infected TB patients is complicated due to adverse drug reactions, drug interactions and less favourable patient outcomes, with an increased likelihood of mortality, lower cure rates and lower treatment success rates compared to non-HIV infected TB patients [101, 102].

Diabetes mellitus

Diabetes mellitus (DM) is reported to be a risk factor for TB, as it weakens the immune responses of the host to bacterial infections. Since the early 20th century, various studies [103] and clinicians have reported an association between DM and TB [104, 105]. Different rigorous epidemiological investigations have revealed that MD is positively associated with TB [106, 107].

Socio-economic and related conditions determining tuberculosis

TB is known to be associated with factors related to socio-economic conditions, including poverty, overcrowding, alcohol consumption, cigarette smoking and malnutrition. Tuberculosis has been known for its link to poverty [108]. For instance, during the industrial revolution of the 19th century, poverty in Europe was accompanied by a high burden of TB and death [109]. Even today, the prevalence of TB is very high among the poor, homeless, displaced, women, drug addicts, elderly and malnourished [82]. In the Western world, following the improvement of nutritional status and housing condition, the risk of TB infection was reduced by 4-6% per annum, and yet the disease disproportionately affects the poor [110-112]. In recognition of this, the diagnosis and treatment of TB is offered for free in developing countries to help reduce the economic burden on TB patients [113].

Moreover, the poor socio-economic condition of the patients and their households could limit access to- and reduce the utilization of the available health services. This may hinder the diagnosis and treatment of TB patients, which subsequently increases the morbidity, mortality and risk of transmission of the disease within the community [114-117]. Under the natural history, where there is no effective treatment intervention, the risk of TB transmission from an infected person to those who are healthy is high in the first two years of infection [118]. Under this scenario, a person with an infectious TB case is likely to infect approximately 10 people, hence generating 20 infected individuals in two years [119]. Moreover, a poor treatment adherence and irregular intake of anti-TB drugs affect the epidemiology of the disease by increasing the period of infection during which one poorly treated TB case could infect 30 individuals [118, 119].

Likewise, in situations where there are no interventions even in the absence of HIV co-infection, about 70% of sputum smear-positive and 20% of culture-positive (sputum-negative) pulmonary TB cases may die within 10 years [120].

It has been a long time since the association between alcohol consumption and the risk of TB was reported [121]. The risk of developing active TB is markedly increased among people who drink more than 40g of alcohol per day and have developed an alcohol disorder. This could be due to an increased risk of infection related to social mixing patterns, which may raise the number and frequency of contacts associated with alcohol use, as well as due to the suppression of the immune system resulting from a high alcohol consumption [121].

Different research has also reported an increased risk of TB infection, and a progression to active TB and TB-related deaths among cigarette smokers [122]. It has been reported that the risk of TB infection and progression to active TB have a dose-response relationship with the number of cigarettes smoked daily and the duration of cigarette smoking [122, 123]. Moreover, a study report from Spain has shown a clear association between TB in children and passive smoking among family members [124]. The association between active TB and cigarette smoking could be due to the adverse effects of long-term exposure to cigarette smoking on the lung's defence mechanisms [122].

Residence

TB patients are more likely to be reported from urban settings due to the overcrowding and high burden of HIV in urban areas compared to their rural counterparts [125]. Additionally, vulnerable and high-risk individuals, such as people with a history of incarceration, alcohol and drug abuse are regularly found in urban settings [126]. This might have contributed to the increased TB case notifications and high burden of the disease in urban settings. In contrast, people in urban settings seem to have a better access to TB care facilities, and could have a better awareness about the disease compared to those in the rural areas.

Nonetheless, the assumed lower risk of TB infection in rural areas may be misleading and should be carefully interpreted among high-TB-burden countries. The less reported number of TB cases from rural settings could be because of limited access to health service, poor health- seeking behaviour and poor housing conditions. Therefore, understanding the TB caseload in rural areas can have substantial implications for the TB control programme in such settings [114, 127].

Tuberculosis laboratory diagnosis

The clinical signs and symptoms of TB are not specific enough. Hence, the use of the laboratory plays a crucial role in diagnosing the disease, monitoring the treatment and preventing transmission. The currently available bacteriologically definitive means for the diagnosis of TB include smear microscopy for acid-fast bacilli using Ziehl-Neelsen (ZN), fluorescent microscopy, mycobacterial culture and a nucleic acid amplification technique known as a polymerase chain reaction (PCR).

Since December 2010, the WHO has endorsed GeneXpert for the simulation detection of *Mycobacterium tuberculosis* (MTB), rifampicin (RIF) for resistance and line probe assay (LPA) for the rapid detection of MDR-TB cases [128]. Radiographic examination (X-ray), especially in the diagnosis of smear-negative PTB [129], and fine needle aspirates for histological examination are also used in the diagnosis of extra-pulmonary TB, mainly for TB lymphadenitis, pleural TB and abdominal TB [130].

Sputum smear microscopy

Sputum smear microscopy that uses direct ZN staining is the most common and preferred diagnostic test for TB. It has been in use for more than a century, and has also played a pivotal role in the successful implementation of DOTS strategy in the globe [131]. In fact, sputum microscopy is the only available method of TB diagnosis at peripheral health facilities having resource constraints.

Moreover, the diagnostic capacity of sputum smear microscopy is suitable in severe PTB cases with a high mycobacterial burden, therefore has a good yield to detect persons with a high risk of disease transmission within a community [132]. It is also one of the key elements of DOTS in diagnosing smear-positive PTB patients, monitoring their response to TB treatment and evaluating treatment outcomes [128].

The major advantages of sputum smear microscopy include its simplicity and reproducibility to use in any setting, as well as its low cost, speed, high specificity and ability to delimit contagiousness [133, 134]. However, the method has some limitations, including its low sensitivity. At least 5,000-10,000 bacilli per ml need to be present in the specimen in order to be detected, although the method cannot be used to diagnose MDR-TB [135]. Sputum smear microscopy that uses acid-fast bacilli (AFB) can diagnose up to 50–60% of PTB cases in well-equipped laboratories, but it has a specificity of over 99% (for *Mycobacterium* Sp. [133, 135].

Consequently, a sputum smear-positive test result is useful in the diagnosis of TB, though a negative test result does not rule out the disease. Moreover, in low-income countries where there is poor access to high-quality microscopy services, there exist lower rates of AFB detection. Furthermore, in countries with a high prevalence of both PTB and HIV infection, the detection rate of PTB using smear microscopy could decline among TB/HIV co-infected cases.

The other limitation of smear microscopy is that presumptive TB cases are usually asked to make repeated visits to a health facility, both to submit sputum and to receive test results. As a result, they could be affected by direct and opportunity costs due to lost work hours and transportation costs, although tests are provided free of charge [136].

In resource-constrained settings, some peripheral health facilities also lack basic infrastructural services such as adequate room, electricity, running water and properly functioning microscopes to provide standardized laboratory services. Additionally, there is a poor supply chain management system to maintain an uninterrupted supply of reagents and chemicals for health facilities, and a scarcity of skilled laboratory workforce in resource-constrained settings.

Fluorescent microscopy

Fluorescent light-emitting diode (LED) microscopy has existed for several decades, and is superior to Ziehl–Neelsen techniques in detecting MTB. For this reason, the use of LED microscopy could increase the diagnostic yield of PTB cases, and is an important tool for an early diagnosis of the disease and improvement in the performance of TB control programmes in low-resource settings [137]. *M. tuberculosis* detection is based on the illumination system of the LEDs, which has more sensitivity compared to ZN smear microscopy [138]. Therefore, examining a smaller number of fields resulted in a higher diagnostic yield compared to ZN microscopy, and reduced the workload of laboratory personnel.

Moreover, the quicker turnaround time might also minimize the diagnostics-related time lost for presumptive TB cases [139]. As result, since 2009 the WHO has recommended the LED fluorescent microscopy phase in as an alternative tool to standard smear microscopy [128, 140]. However, the major challenges associated with fluorescent microscopy are its dependence on electricity and its unstable supply of reagents, especially in peripheral health facilities located in resource-poor settings [141]. Like ZN microscopy, fluorescent microscopy also does not detect MDR-TB.

Bacterial culture

Mycobacterial culture has been widely accepted as a gold standard to ensure a definite diagnosis of TB, as it is the accepted method in assessing patient follow-up and confirming a cure. Consequently, liquid culture has been established as a standard for TB diagnosis in high-income countries [142]. Sensitivity of liquid culture in the detection of TB is 10% higher than solid culture. Moreover, both liquid and solid cultures are highly sensitive in detecting *M. tuberculosis* compared to ZN and ELD microscopy.

The major features of culture that made it the gold standard for the diagnosis and follow-up of TB include its sensitivity to detect as few as 10 bacteria per millilitre compared to smear microscopy, which can only detect 5,000-10,000 bacteria per millilitre. It also has the ability to correctly identify the isolated strains, and provide a definitive confirmation of negative conversion and the healing of patients under treatment [143].

Even so, it has some limitations, including the long time elapsed from sample receipt to the reporting of the result (about four to six weeks in conventional solid media), its high cost compared to smear microscopy, the specific media it requires with subsequent storage in a hotplate and the specific training of personnel it requires to perform cultures. Moreover, culture is largely dependent on previous steps of sample decontamination and digestion to help destroy the undesired bacteria that would allow the mycobacteria to survive and access nutrients for growth [143]. Other drawbacks include laboratory cross-contamination, which may lead to false-positive results [144, 145], and a long incubation period that delays the beginning of treatment, and consequently increases the risk of mortality and transmission of the diseases within the community [145]. In low-resource settings, the stringent bio-safety requirements of culture can only be met at referral laboratories, and this might limit the accessibility of culture services at peripheral health facilities where the majority of the population reside.

Moreover, the weak supply chain management system of those poor resource settings may not have in stock the required reagents and chemicals for culture. Culture tests demand trained and skilled laboratory personnel, who are often scarce in low-income countries. Therefore, bacterial culture services as a primary diagnostic tool for TB may remain unrealistic in such a setting.

PCR

Polymerase chain reaction (PCR) amplifies a specific segment of *M. tuberculosis* deoxy-ribonucleic acid (DNA). In high-income countries, it has been commonly used for the diagnosis of TB for over a quarter of a century. Although its use has improved the diagnosis of TB and patient care, it has a different diagnostic performance due to its wide sensitivity ranging between 10%-90% [146].

The GeneXpert MTB/RIF assay was considered as an advanced diagnostic tool for detecting TB and MDR-TB [147]. In 2010, the WHO recommended and endorsed the tool for the primary diagnosis of HIV-associated TB and the detection of MDR-TB cases [148]. Since 2014, the diagnostic use of GeneXpert MTB/RIF assay has been extended to the diagnosis of extra-pulmonary TB in adults and TB in children [128].

In addition to its usefulness in the detection of MDR-TB, GeneXpert MTB/RIF is a fully automated and simple modular system that can be used by existing and minimally trained staff. As compared to culture, the risk of contamination of GeneXpert MTB/RIF is low. Stringent bio-safety requirements are not mandatory, and what is intended for smear microscopy is sufficient for the GeneXpert MTB/RIF TB testing laboratory. According to a Cochrane review of the diagnostic performance of the GeneXpert MTB/RIF assay method, there was a pooled sensitivity of 88% among HIV-positive adults [149], which is high compared to the 50-60% sensitivity of ZN smear microscopy [149].

Nonetheless, MTB/RIF assay has some limitations, such as missing nearly half of smear-negative culture-positive TB cases [150]; and depending on the prevalence of MDR-TB, a low proportion of rifampicin resistance false-positive has been reported [151]. Moreover, due to the high initial cost of the instrument and disposable cartridges, it is not affordable as an alternative tool of TB diagnosis in resource-constrained settings. Because Gene Xpert MTB/RIF requires an uninterrupted power supply to maintain an ambient temperature at 28^{o} C or lower, it is also not feasible for use at peripheral health institutions where there is either an unstable or no electric power supply [149].

In the absence of qualified biomedical engineers, the annual calibration of the GeneXpert MTB/RIF assay could also be a challenge at peripheral health institutions [152]. Hence, using GeneXpert MTB/RIF at district or sub-district health facilities as recommended by the WHO, is primarily a challenge in poor resource settings.

Chest radiography

Chest radiography has been used as a supportive method for TB diagnosis [153]. However, X-ray does not provide a definitive diagnosis, and has a lower specificity than other methods. It is usually recommended to be supported with other diagnostic methods [154]. In HIV-positive individuals, radiologic findings of PTB are frequently similar with other pulmonary diseases such as pneumonia. In such patients, radiologic findings are non-specific, particularly in advanced immuno-compromised HIV-positive individuals. In low-income countries, X-ray facilities are therefore often restricted to major hospitals.

Tuberculosis treatment

The goal of TB treatment is to confirm a relapse-free cure and prevent the emergence of drug resistance. Because of this, the effect of treatment should not be judged solely by the anatomical healing of lesions, but also by the sterilization and elimination of bacilli from the sputum. *Mycobacterium tuberculosis* is known for its slow growth and ability to remain dormant for a long period. Therefore, a prolonged treatment using multiple drugs is required to guarantee a relapse-free cure and prevent the emergence of resistance [155].

An effective TB treatment is intended to cure the patient, prevent deaths from active TB or its late complications, prevent the emergence and spread MDR-TB, minimize relapse and protect the community from an ongoing transmission of the disease. All TB treatment regimens have both an initial intensive phase and a continuation phase [155, 156].

The initial intensive phase of TB treatment is intended to kill actively growing and semi-dormant TB bacilli. Thus, the intensive phase treatment can shorten the duration of infectiousness by rapid smear conversion (80–90%) following two–three months of treatment. The treatment regimen of intensive phase lasts for two months for new TB cases, and involves a fixed-combination dose of four drugs, including isoniazid (H), rifampicin (R), pyrazinamide (Z) and ethambutol (E) (2HRZE), followed by the continuation phase [155].

Tuberculosis patients with a previous history of TB treatment are more likely than new patients to harbour and secrete bacilli resistant to anti-TB drugs. Therefore, the initial intensive phase treatment of such TB cases should consist of five drugs with at least two effective drugs to reduce the risk of selecting additional resistant bacilli [155]. The intensive phase for retreatment cases (relapse, treatment interrupted and failure) lasts for three months and involves five drugs, including 2HRZES (streptomycin(S)) for two months, and four drugs (2HRZE) in the additional month. The rapid killing of tubercle bacilli in the intensive phase could convert infectious TB cases to non-infectious within a few weeks. As a result, most sputum smear-positive TB cases become smear-negative within two months [155].

The continuation phase treatment lasts for four to six months, with its primary objective being to sterilize and eliminate the remaining bacilli so as to reduce the chance of relapse and treatment failure [157]. Treatment results include treatment completed or cured (treatment success), lost-to-follow-up (defaulted), treatment failure and death from any cause while on treatment (death). These are indicators used for monitoring the performance of DOTS and TB control programmes [86]. Treatment failure and lost-to-follow-up are indicators to measure a poor management of TB treatment. Effective TB treatment is very valuable in TB control programmes, as it contributes to the prevention of the emergence of drug resistance and spreading of the disease in the community, as well as reducing mortality due to the disease [155].

Global situation of tuberculosis

Tuberculosis (TB) remains a high-priority communicable disease that causes an enormous burden of morbidity, mortality and infected an approximately one-third of the global population, and is the second leading cause of death among infectious diseases worldwide [94, 100, 158, 159].

However, there is a high variability in the burden of TB around the world, with 58% of the disease occurring in Asia and 28% in Africa, whereas a smaller proportion of the disease occurs in the eastern Mediterranean region (8%), the region of the Americas (3%) and the European region (3%). The African region had the most severe burden, with 281 incidents of TB cases per 100,000 population on average, which is more than double the global average of 133 cases per 100,000 population [86].

The African region, especially Sub-Saharan Africa, has the highest proportional rate of TB cases and deaths when its small population is compared with that of the world. In 2013, one-fourth of global TB cases were reported from Sub-Saharan African countries. Moreover, during the same year, from the 10 countries that contributed three-fourths of the 3.6 million estimated missing cases, five countries were from Sub-Saharan Africa [128].

In general, the disease disproportionately affects people in resource-poor settings, particularly those in Asia and Africa, as more than 80% of TB cases and 78% of deaths occurred in developing countries [128, 160, 161]. However, high-income countries such as Western Europe, Australia, New Zealand, Canada and the United States of America have less than 10 TB cases per 100,000 population per year, whereas the high-burden countries (HBCs) have rates ranging from 150 to 300 cases per 100,000 population [86].

The 22 high-burden countries accounted for 83% of the overall estimated TB incident worldwide. Of these 22 countries, the six countries with the largest number of incidents in 2014 were India, Indonesia, China, Nigeria, Pakistan and South Africa, with Bangladesh, the Philippines, DR Congo and Ethiopia rounding out the top 10 in terms of the number of TB cases (2014 WHO). In the same year, there were 9.6 million TB incidents, including 1.2 million (12%) cases co-infected with HIV and 1.5 million deaths around the globe [86]. The proportion of TB-HIV co-infection was highest in the African region, where approximately 32% of TB cases were co-infected with HIV, which accounted for 74% of global TB-HIV co-infection in 2014 [86].

Multidrug-resistant TB (MDR-TB) is defined as *Mycobacterium tuberculosis* strain resistant to at least the first-line anti-TB drugs, Isoniazid and Rifampicin [159]. MDR-TB can be developed either when a person is infected with a resistant strain, or when an insufficient or improper treatment leads to a drug selection of the resistant strain [159]. When a person with no history of first-line anti-TB treatment develops MDR-TB, it is known as primary resistance MDR-TB, while when a person with a history of first-line anti-TB treatment acquires MDR-TB, it is called secondary resistance MDR-TB [162].

The emergence of MDR-TB has posed challenges to the global TB control programme efforts [86, 159]. Even though efforts were made in the identification and treatment of MDR-TB globally, the majority of the cases have not yet been diagnosed. Globally, approximately 3.3% of new TB cases and 20% of previously treated ones were estimated to have MDR-TB. The prevalence of resistant TB to one or more drugs and MDR-TB varied significantly across different countries, though more than half of the global MDR-TB cases are found in India, China and the Russian Federation [159].

The overall global number of estimated MDR-TB cases in 2014 was 480,000, with 190,000 of these dying in the same year. Of the total estimation, only 123,000 (26%) had been diagnosed, 111,000 (23%) had access to second line anti-TB drugs and only 50% of them were successfully treated [86].

Moreover, the increasing number of XDR-TB is a serious global concern. In fact, 9.7% of MDR-TB cases are estimated to have XDR-TB, and in 2014, XDR-TB cases were reported from 105 countries [86]. The number of countries that reported XDR-TB was only 40 in 2011, but that increased to 100 in 2013 and 105 in 2014. XDR-TB is resistant to any fluoroquinolone, and to at least one of the three second-line injectable anti-TB drugs - kanamycin, capreomycin and amikacin - in addition to isoniazid and rifampicin. XDR-TB is also associated with high risk of mortality [163-165].

The main causes of TB epidemic in the world are socio-economic conditions, an increase in human immuno-deficiency virus (HIV burden) and an increase in anti-TB drug resistance, especially in relation to the MDR-TB strain. Moreover, the weak and ineffective national TB control programmes, the poor implementation of infection prevention measures, a poor quality and accessibility to anti-TB drugs, the irrational use of anti-TB treatment regimens and the poor patient adherence results in the emergence of MDR-TB and extremely drug-resistant (XDR) TB, which is a global challenge in TB control programmes [166, 167].

Global tuberculosis control

Over the past 60 years, following the discovery of anti-TB chemotherapeutic agents for the combined treatment of TB, the primary efforts were to find an effective strategy to control tuberculosis. As a control effort, the international community and the WHO exercised a vertical TB control programme in most parts of the world over the years between 1948 and 1963. However, the programme was found to be ineffective against the increasing challenges of the TB epidemic in low-resource countries [168].

Furthermore, Bacille Calmette-Guérin (BCG) has mainly been used as a supplement in TB control efforts in high-TB-burden countries to reduce the disability and death resulting from TB meningitis in young children [76, 169, 170]. Nevertheless, its protective efficacy against TB in the adult population, and its impact on TB transmission, is inadequate [169, 171].

The ultimate goal of TB control is to eliminate the disease from the population by reducing the risk of transmission of *M. tuberculosis* and eventually doing away with the disease [76]. The main strategy to control TB is the prompt detection and curing of infectious cases, as the effectiveness of TB control strategy primarily depends on the timely diagnosis and treatment of smear-positive pulmonary tuberculosis. In fact, the cure of smear-positive pulmonary TB patients is considered to be an important intervention mechanism for the primary prevention and emergency of MDR-TB. Hence, the rapid identification of smear-positive pulmonary TB cases and their effective treatment using combined anti-TB drugs is a cornerstone in the global TB control programme [76].

In 1993, the WHO declared tuberculosis to be a global public health emergency and recommended the Directly Observed Treatment, Short Course (DOTS) as a standard strategy to control the disease [172, 173]. In 1994, the WHO designed a Framework for Effective Tuberculosis Control [174], which clearly designated the core elements of the DOTS Strategy. The DOTS Strategy has five key elements considered to be essential for global TB control [174]: political commitment; detection of infectious TB cases using sputum smear microscopy; standardized case management with short-course chemotherapy under direct supervision during, at least, the intensive phase; the uninterrupted supply of anti TB drugs, and proper registration,

record keeping and reporting mechanisms, including the follow-up of treatment outcomes [175] . DOTS had the aim of detecting 70% of infectious cases and successfully treating 85% of them to interrupt the transmission, reduce mortality and prevent the emergence of drug resistance by the year 2005 [172, 173]. In 2006, the DOTS strategy was revised as a Stop TB Strategy to dramatically reduce the global TB burden, in line with the Millennium Development Goals (MDGs), which aimed to decrease the prevalence and death resulting from TB by 50% in 2015 [176, 177].

The MDG target to halt and reverse TB incidence by 2015 was achieved worldwide, and in 16 out of the 22 high-TB-burden countries (HBCs). The TB incidence rate declined by 18% between 2000 and 2015, with a 1.5% annual rate of reduction between those years. This rate of reduction is not sufficient for reaching the global target of TB elimination set as a target in the Sustainable Development Goals (SDGs), thereby possibly warranting the need for intensified efforts to control the disease.

Globally, the achievements in the reduction of TB mortality and the TB prevalence rate were lower in 2015 by 47% and 42%, respectively, compared to those in 1990. Different TB control efforts implemented in different settings contributed to early case detection, prompt treatment and a reduction in disease burden. Decentralizing TB care to the community, collaboration between public and private health-care providers, and TB/HIV collaborative activities, were some of the efforts employed to achieve the intended MDGs TB control.

Nonetheless, the global target of a 50% reduction in TB mortality and TB prevalence has not yet been achieved. All three MDGs of TB control targets were met in the Americas, the Western Pacific region, Southeast Asia, and nine of the of 22 HBCs, including Ethiopia. Still, achieving the MDGs in TB mortality and TB prevalence was not possible in Africa and Europe with the exception of Western Europe. Overall, the implementation of the DOTS strategy has saved the life of 35 million TB patients between 2000 and 2014, and 43 million between 1990 and 2015 [86]. Even so, there are still challenges that remain in funding TB control efforts, 3.6 million undiagnosed TB cases every year, with only 25% of MDR-TB cases detected and only one in two MDR-TB cases cured [178].

Although mortality due to TB and TB prevalence has declined globally by 47% and 42%, respectively, between 1990 and 2015, TB remains at the top of the public health agenda after the MDGs. In 2014, the World Health Assembly endorsed the 2015 Post-sustainable Development Goals (SDGs) of TB control targets to help reduce the number of TB deaths by 90%, the TB incidence rate by 80% and zero families facing catastrophic costs due to TB by 2030 compared with 2015 [86, 128].

Integrated, patient-centred care and prevention, bold policies, supportive systems and intensified research, as well as innovation, are the main pillars and components of the strategies of SDGs [86]. The achievement of these goals warrants a continued effort in both low- and high-incidence countries towards controlling the disease [179, 180]. The SDGs' plan to end the TB epidemic is to achieve an average reduction of TB incidence by 5% per annum until 2025, and then by 4% per annum, in order to reach the 2035 global target [86].

Nonetheless, the annual average reduction of TB incidence of 1.5% between 2000 and 2014 was inadequate, and the trend in reduction was far from the intended target to achieve global TB elimination [86]. Thus, universal access to better diagnostics, including DST, high-quality safer, easier and shorter treatment regimens and research, are all recommended to achieve the SDGs' target. In this regard, operational research needs to be intensified in order to identify the gaps, find applicable solutions to TB control, and provide evidence on the epidemiology of the disease in targeting high-risk populations for targeted interventions.

Tuberculosis in Ethiopia

Country background

Ethiopia is one of the oldest countries in civilization in the world. It is the oldest independent and second most populous country located in East Africa, and shares borders with Eritrea in the north, Djibouti in the east, Somalia in the east and southeast, Sudan and South Sudan in the west and Kenya in the south and southeast. Ethiopia stretches over an area of 1.1 million square kilometres, with a geographical diversity ranging between 4,620m above sea level at Ras Dashen down to the Danakil (Dallol) Depression of 148m below sea level [181]. The country has a population of 90 million [182] with a variety of nations, nationalities and peoples speaking over 80 different languages. It has a unique cultural heritage with a diversified population mix of ethnicity and religion [183].

Administratively, the country is divided into nine regional states and two city administrations. Each regional state is then further divided into zones (second level), and then woredas or districts (third level) and kebeles (fourth level). The *kebele* is the smallest administrative unit, with an average population of 5,000 people. Currently, there are a total of 94 zones, 817 *woreda*s (districts) and 16,253 *kebeles* in Ethiopia [184].

Approximately 83% of the Ethiopian population resides in rural areas [185], with a majority of this population living in the highlands on subsistence farming. About 10%, however, live in the lowlands of the south eastern and eastern parts of the country on pastoralism. Ethiopia's GDP has doubled over the past 12 years [186]. The current annual per capita income of the country is USD 470 [182], with a per capita health expenditure of USD 20.8 [187].

During the last two decades, the life expectancy in Ethiopia has risen to 63 years [182]. The population structure is predominantly young, in which 44% are children under 15 years of age and 53% within the age group between 15 to 65 years, with only 3% of the total population over the age of 65 years. The country also has a total fertility rate of 4.1 [188]. According to the 2013 FMOH health and health-related indicator report, the physician-to-population ratio stands at 1 per 20,970 population [187]. Similar to many other low-income countries, communicable diseases are the major health problem within the country. However, in recent years, the prevalence of non-

communicable diseases, such as cardiovascular diseases, diabetes mellitus and cancer, is on the rise [189].

Health system and health-related problems in Ethiopia

The existing Ethiopian health policy aims at improving access to- and the quality of health services. The priority of the policy is to prevent and control communicable diseases, and promote and provide basic curative services to ensure an equitable and acceptable access to the health services [190, 191]. In Ethiopia, HIV, tuberculosis, malaria, malnutrition, acute respiratory tract infection, helminthiasis and dysentery are the top leading causes of morbidity [187].

The country has established a three-tier health-care delivery system, in which the primary health-care level is designed to include one district hospital, four health centres and 20 health posts on average. The secondary level comprises general hospitals, while the tertiary level consists of specialized teaching hospitals. More specialized clinical care services are provided at general and specialized hospitals, whereas comprehensive clinical care and preventive and promotive health services are given at the primary health-care level. The level of health facilities with health-care providers, and the size of population they serve, are summarized as follows.

The government has introduced a new innovative community-based approach known as the health extension package (HEP). This package has been implemented for over a decade with the primary aim of ensuring access and equity health care at the community level [192]. Each *kebele,* the lowest administrative unit in the country, has one health post run by two health extension workers to serve a population of 3,000-5,000 in the rural areas [192, 193]. Currently, more than 16,000 health posts are found all over the country, and in 2012, approximately 38,000 health extension workers were deployed nationally to provide preventive, promotive and basic curative health services in the rural communities. Moreover, in the urban areas, nurses trained in health extension packages have been deployed to provide preventive and basic curative health services [193].

In recent years, the country has given more attention to the expansion of health facilities to help improve access and ensure universal basic health service coverage. In 2014, there were 16,251 health posts, 3,335 health centres and 156 public hospitals providing services in Ethiopia. In the same year, the physician-population ratio was 1:20,970 with a disproportionate distribution. The majority of the physicians worked in major towns.

Following the implantation of Health Sector Development Plans for the past 20 years, Ethiopia introduced a Health Sector Transformation Plan (HSTP) in line with the country's Growth and Transformation Plan II of 2015. The HSTP gives priority to the prevention and control of communicable diseases including malaria, HIV and TB, as well as improving health service quality. The TB control plan in HSTP is in line with the global End TB strategy, with the aim of decreasing mortality and morbidity from TB. The plan primarily gives due attention to improving case detection, providing diagnostics facilities, maintaining a higher treatment success and addressing drug-resistant TB and TB/HIV collaborative interventions [181].

Through the implementation of the health extension package and primary health care, Ethiopia has made significant improvements in maternal and child health [186] and a reduction in the burden of communicable diseases [194]. As a result, the country has achieved the MDG targets in the reduction of child mortality and deaths resulting from malaria, TB and HIV [195], with Table 1 showing some Ethiopian health and demographic indicators:

Table 1: Indicators of Ethiopian health and demographic status

Indicators	Year	National estimates	References
Population	2014	90 million	[187]
Life expectancy	2014	63 years	[187]
Total fertility	2014	4.1	[188]
Gross domestic product (GDP)	2014	470 USD	[187]
Physician-to-population ratio	2013	1 per 20,970 population	[187]
Child mortality rate	2013	68/1,000 live births	[195]
Maternal mortality ratio	2013	350 per 100,000 live births	[195]
Annual TB incidence	2013	224/100,000 population	[128]
Smear-positive TB prevalence	2011	108 per 100,000 population	[196]
Proportion of multi-drug resistance TB cases	2013	1.6% among new and 12% among retreatment cases	[128]
Adult HIV prevalence	2013	1.5%	[197]
Proportion of TB-HIV co-infection	2015	23%	[86]

National tuberculosis control efforts

In Ethiopia, the efforts of TB control began in the early 1960s with the establishment of a few TB centres and sanatoriums in some urban areas. However, the control efforts were vertical and not well coordinated until 1976 when the national TB control programme office was established [198].

TB control aims at detecting infectious TB cases as early as possible and putting them on standardized anti-TB treatment in order to cure and break the chain of transmission and to avert the emergency of multi-drug resistance. In order to attain these aims, the health policy of Ethiopia has given priority to the control of TB since 1993 [190], and the TB control programme has been progressively decentralized under the country's primary health care strategy. In 1994, the Federal Ministry of Health combined the TB and Leprosy Control Programme (TLCP), and integrated it into the general health services [199]. The main objective of TLCP is to ensure the provision of quality diagnostic and treatment services integrated within the existing health-care system.

The WHO-recommended DOTS strategy was piloted in the Arsi and Bale Zones of Ethiopia in 1992, and was then launched in 1995 in a few health facilities [199]. Since then, the country has expanded and decentralized DOTS to public health facilities. The aim of the DOTS strategy is to detect and treat infectious TB cases as effectively as possible, and help curb the spread of the disease within the community [199, 200]. In 2014, approximately 4,577 health facilities, including 137 private health institutions, were implementing DOTS services. Even so, private health facilities are few in number, and contributed only 14% of the total notification during the same year.

Ethiopia is among both the 22 high-TB-burden countries and the 27 high-MDR-TB-burden countries worldwide [181]. TB is the third leading cause of hospital admissions and the second top cause of death in the country [128, 199].

According to the 2015 WHO report, the annual incidence of all forms of TB cases in Ethiopia is estimated to be 207, while that of prevalence is 200 per 100,000 people. These figures are higher than the respective estimates of 174 and 133/100,000 people in the world (WHO, 2015). In 2014, the TB case notification for all forms of TB cases was 123 and 43.3 per 100,000 people for smear-positive TB in Ethiopia [128].

Ethiopia also carried out a national TB prevalence survey for the first time in 2011, and found out prevalence rate of 108 smear-positive TB cases per 100,000 people [196]. However, different study reports from various parts of the country showed that the prevalence of smear-positive cases ranged from 33 to 213.4/100,000 people in Ethiopia [201-204]. This might indicate the presence

of variations in TB burden across different parts of the country. According to the National TB Control and Prevention report, there is also a variation in TB case detection rates and notifications across different parts of the country [187]. Thus, understanding the epidemiology of the disease and monitoring DOTS performance could help in devising area specific targeted TB control strategies.

Ethiopia is also one of the 27 high-MDR-TB-burden countries in the world, in which its proportion among new and retreatment cases was 1.6% and 12%, respectively [86]. In 2014, a total of 2,405 MDR-TB patients were diagnosed and enrolled for second line anti-TB treatment in the country [86]. Since 2012, a community-based active TB case finding has been implemented through the involvement of health extension workers, and has contributed to the early case detection and improvement of TB treatment outcomes in Ethiopia [205]. Health extension workers provide health education to the community to help improve overall awareness about TB. Furthermore, they are engaged in house-to-house visits to identify presumptive TB cases and refer them to provide sputum for smear microscopy and treatment follow-up. This strategy has improved access to TB care and contributed to early case detection and TB treatment outcomes, with an overall enhancement of DOTS performance in Ethiopia [181].

Following the 2011 global Stop TB Plan, substantial progress has been made in accessing laboratory services [187], in which a total of 2,107 smear microscopy, 23 GeneXpert MTB/RIF and seven cultural and DST laboratory services were established and now provide laboratory services in the country [128]. Although Ethiopia has met TB-related MDG, it still is one of the top 10 high-TB-burden countries in the world, and has contributed 3% of the 3.6 million estimated missing diagnosed TB cases across the globe [128].

The rationale for the study

The intended goals of the TB control programme are to decrease morbidity and mortality due to the disease and to prevent its transmission, together with the development of drug resistance. These goals could be attained through a well-planned and implemented TB control programme [89, 206]. However, there is often a lack of necessary information for proper planning and evidence-based decision making in resource-constrained settings.

Approximately 80% of TB cases and 78% of global TB deaths occurred in Sub-Saharan Africa, which is mainly due to the high prevalence of human immuno-deficiency virus (HIV), poor TB control efforts, social inequalities, drug resistance and inadequate access to TB care [160, 161, 207].

The WHO recommended the DOTS strategy to promote passive case findings with the provision of standardized anti-TB drugs treatment. However, this strategy relies on the patients' willingness to present themselves to TB clinics for an evaluation of their symptoms. In this case, it is assumed that TB patients are knowledgeable about the symptoms of the disease, and have free access to TB care without any socio-cultural barrier.

Nevertheless, passive case findings mainly serve those who are better in their knowledge about the disease, have health-seeking behaviour and have better geographic access to the services. The DOTS strategy may be difficult to implement on patients who do not have access to TB care services, and such circumstances may lead to delays in diagnosis and treatment. This may in turn increase the risk of disease transmission [114, 208]. Hence, there should be reliable information to seek a workable alternative intervention for policy makers and TB programme managers for effective control of the disease.

Over the past two decades, the implementation of DOTS strategy has improved the number of identified and treated TB cases. However, despite the decentralization and increase in the number of DOTS sites in the country, the number of diagnosed TB cases is still far below the expected target. This could be due to an overestimated number of expected TB cases in the community, underreported the diagnosed cases or due to a reduction in TB incidence as a result of DOTS expansion. However, in the absence of good epidemiological indictors such TB incidence and

prevalence, one cannot measure the real impact of DOTS strategy in TB control efforts [209-211] or the actual TB burden in the community. Moreover, the true TB prevalence and incidence at the community level could only be obtained through both population-based surveys and prospective follow-up studies [210].

Yet, these kinds of data are lacking in most developing countries [201, 212]. As a result, the TB control programmes in resource-constrained countries, including Ethiopia, often lack reliable information required for proper planning and evidence-based decision making. Moreover, most of the information used for such purposes, particularly in relation to TB notification and treatment outcomes, is obtained from health facilities, but often lacks completeness and consistency [201].

Consequently, a population-based survey to determine the prevalence of smear-positive pulmonary TB and multi-drug resistance, and a follow-up study to measure TB incidence, are all critical pieces of information to better understand the epidemiological dynamics of TB and strengthen the control efforts. Obtaining scientifically sound baseline information on the true TB burden and regularly analysing trends on morbidity, mortality, case-notification and treatment outcomes could help to fill the information gap in TB control programmes. This study was therefore carried out to help bridge these gaps. Figure 1 shows a schematic model of the pathogenesis of the disease and the clinical course of TB with the study topics covered in this thesis.

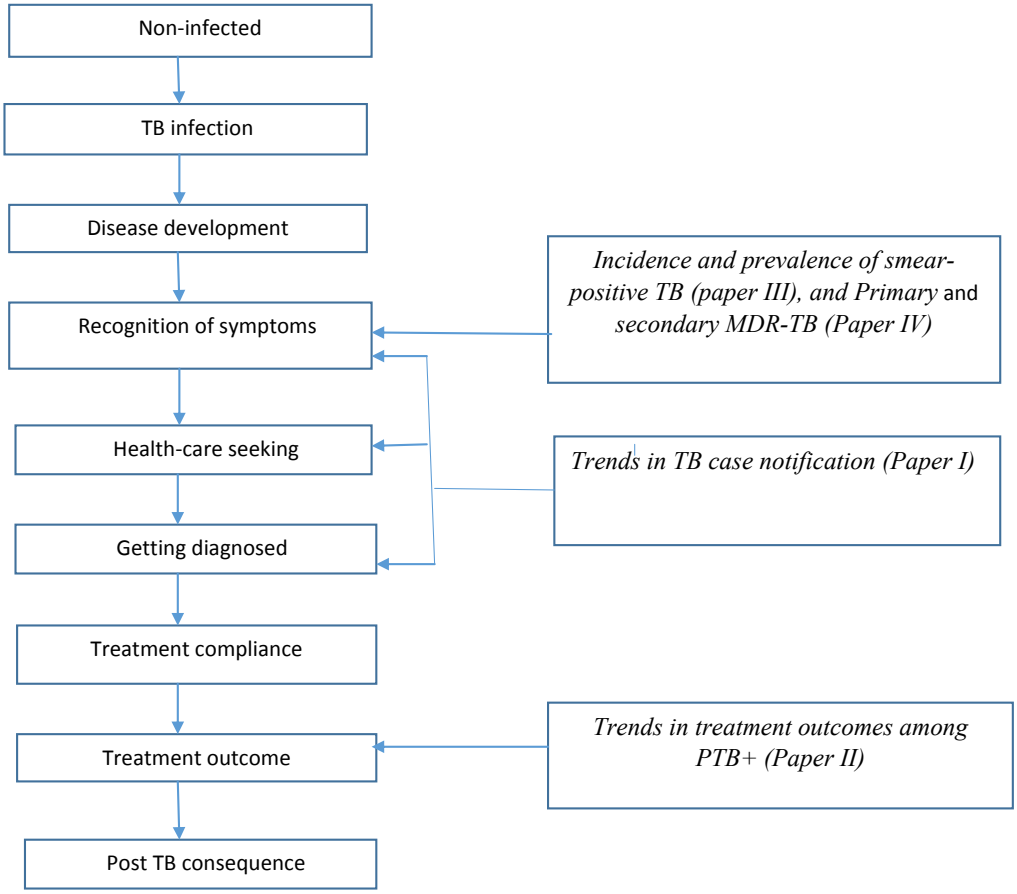


Figure 1: A schematic model of the natural history of TB in relation to the study objectives

The thesis therefore focuses on the analysis of TB control performance, and attempts to estimate the TB burden to improve TB control programmes in Ethiopia. TB case detection and treatment outcomes are determined by individual, social and biomedical factors [114, 213, 214]. Low TB case detection should be a serious concern from both public health and individual patient points of view. As discussed earlier, one untreated infectious TB case could on average infect roughly 20 individuals over a two-year period [215], and 50% of undiagnosed and untreated TB cases die within five years of the start of symptoms. Furthermore, 25% are cured spontaneously, while the remaining 25% continues to transmit the disease [216]. Thus, a low TB case detection is a serious

concern in attaining the global target in most of the resource-constrained countries, including Ethiopia [86, 217, 218]. As a result, there is a need to study the trends in TB case notification, which was covered by Paper I in this thesis.

Similarly, a low success in treatment completion is another important challenge, as a poor TB treatment outcome is a risk factor for the emergence of multi-drug resistance. Moreover, poorly treated TB cases could have a longer infection period and infect, on average, 30 individuals, and 30% of those inappropriately treated PTB+ cases die [219]. For this reason, there is also a need to study the trends in TB treatment outcomes to better understand the determining factors to design an evidence-based alternative strategy to improve TB control programmes in Ethiopia (Paper II).

The true TB incidence used to estimate the annual expected number of TB cases and utilized for planning purposes in most developing countries is often derived from TB case notifications, with expert opinions estimating under-reporting and under-diagnosis. This is often used to form the national TB prevalence survey [86]. However, using such a method to estimate TB incidence requires a good health-care system in TB case notification, with both good recording and reporting. Such a health-care system is lacking in most resource-constrained settings.

Deriving TB incidence from the TB prevalence survey also requires a good estimate of the disease duration, which is difficult to measure based on prevalence surveys. As a result, the actual TB incidence in most developing countries, including Ethiopia, is not well known. For this reason, a well-designed and comprehensive population-based study to help estimate the prevalence of smear-positive pulmonary TB, multi-drug resistance and TB incidence at community level is valuable in generating reliable data that can help fill the gap in the epidemiological dynamics of TB. Obtaining such data on the epidemiology of TB is critical for policy makers to make evidence-based decisions and develop proper planning to improve TB control programmes.

Hence, we did population-based studies to measure the prevalence and incidence of smear-positive pulmonary TB (Paper III) and primary and secondary multi-drug resistance (Paper IV). This study might be the first attempt in carrying out estimates of primary and secondary drug resistance and among very few studies that estimates the incidence of bacteriologically confirmed pulmonary TB cases (Paper IV) at the community level in a developing country.

In general, the study topics in this study were designed to measure DOTS performance in TB case notification and their treatment outcomes, and also measure the actual burden of TB at a community level.

Objectives

General objective

The overall aim of this thesis is to assess the trends in TB control performance and estimate the burden of the disease at the community level for a better understanding of the gap in improving the TB control programme in Ethiopia.

Specific objectives

i. To evaluate a 15-year trend in TB case notification in relation to DOTS expansion (Paper I)

ii. To evaluate a 15-year trend in treatment outcomes in relation to DOTS expansion (Paper II)

iii. To estimate the prevalence and incidence of smear-positive pulmonary tuberculosis and the burden of undiagnosed TB cases in the community (Paper III)

iv. To estimate the primary and secondary Anti-TB Drug Resistance at the community level (Paper IV)

Methods

Study setting and population

The studies were conducted in the Oromia Regional State, which is the largest and most populous in Ethiopia. It is located in the central part of the country, and shares international borders with Somali and Kenya. It covers 359,619.8 km^2 (30% of the national area), and has a population of 35 million (35% of the national population). The regional state has 20 zones and 16 city administrations. Approximately 89% of its population lives in rural areas, and about 51% are males. The basic health service coverage and health service utilization rate is 90% and 30%, respectively, and the DOTS service is given at all hospitals and health centres of the region.

The studies were conducted specifically in the Arsi Zone, Oromia Regional State. The Arsi Zone is located 175 kms to the southwest of Addis Ababa. It is one of the 20 zones of the region, and has one urban and 24 rural districts. The zone has a population of 3.1 million people in an area of 21,120.28km^2. It is one of the most densely populated zones with 148 people per km^2 [220].

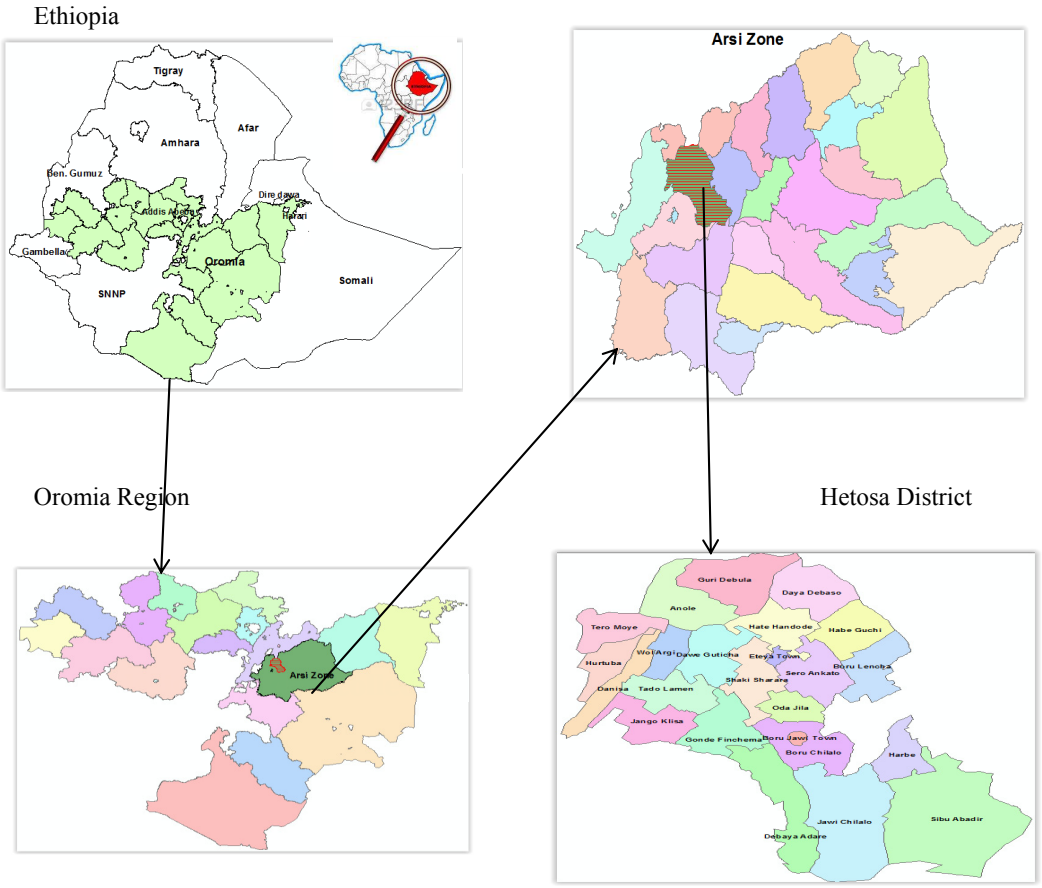
In 2015, approximately 89% of the population lived in rural areas, while 92% of the zonal population lived within a two-hour walking distance from a public health facility. According to the national standard, any part of the population living within a two-hour walking distance or 10 km radius of either a health centre or a hospital is considered to have access to DOTS and other health services [221]. Moreover, a hospital or health centre with DOTS service during the study period was considered as a DOTS site. Accordingly, the DOTS population coverage of each district of the zone was computed by taking the population living within a two-hour walking distance or 10 km radius (estimated at 250,000 for a hospital and 25,000 for a health centre) of a hospital or a health centre as a nominator, and the total number of mid-year population of each district as denominator multiplied by 100.

The zonal and regional Tuberculosis and Leprosy Control Programmes (TLCP) were set up in 1994 when the national TB control programme was reorganized. Before the programmes were set up, there were vertical TB control programmes that were centrally coordinated. In 1992, a DOTS pilot project was carried out under the National TB Control Programme in a number of districts in

the Arsi Zone, of which Hetosa was one of such districts. Since 1997, the DOTS programme has gradually expanded, and was integrated into the general health services of the zone. In 2011, all health facilities of the zone were used for TB diagnostic and treatment units, with standard TB Unit registers approved by the National Tuberculosis and Leprosy Control Programme.

Hetosa is one of the 25 districts of the Arsi Zone, Oromia Regional State and Central Ethiopia. It is a district that typically represents all of the features of the Arsi Zone in terms of its population density, socio–economic state and demographic condition. The DOTS and health service coverage, the proportion of urban-rural population and HIV prevalence, and TB case notification and treatment outcomes of the Hetosa District, are also similar to that of the entire zone. The district has an estimated population of 178,229 living in 23 rural and one urban *kebele* (the smallest administrative unit in the government structure) [205]. Since 2010, *kebeles* have been further divided into three sub-*kebeles* known as *gote.* Each *gote* has about one-third of the population of a *kebele* [205].

Figure 2 - Map of the study area



This thesis is based on a combination of cross-sectional and prospective cohort study designs. In Papers, I and II, we employed a facility-based cross-sectional study design on the cohorts of TB cases registered over 15 years. In Paper I, all forms of TB cases (smear-positive, smear-negative and extra-pulmonary), and in Paper II, all smear-positive TB cases registered in all DOTS-providing health facilities over the 15 years, were included.

In Papers III and IV, we employed community-based cross-sectional- and prospective cohort study designs. We employed a cross-sectional study at baseline to measure the prevalence of smear-positive TB cases and a prospective follow-up study to identify the incidence of smear-positive TB cases (Paper III). In Paper IV, we employed a cross-sectional survey at baseline and subsequent surveys to measure the burden of primary and secondary anti-TB drug resistances of the study area. A comprehensive description of the study designs and sampling procedures has been presented in individual papers, with the study designs and the study population summarized in Table 2 below:

Table 2: Summary of the study designs and study population

Paper	Topic addressed	Design	Study population	Study period
I	Trends in TB case notification	Cross-sectional, Retrospective trend analysis	41,965 all forms of TB patients registered between 1997 and 2011	January to March 2013
II	Trend in treatment outcomes among PTB+	Cross-sectional, Retrospective trend analysis	14,221 PTB+ patients registered between 1997 and 2011	January to March 2013
II	Incidence and prevalence of smear-positive TB	Cross-sectional and Prospective cohort	33,073 adults surveyed at baseline and 32,800 individuals at follow-up study	July 2013 to June 2014
IV	Primary and secondary MDR-TB	Cross-sectional with subsequent surveys	33,073 adults surveyed in a rural district	July 2013 to June 2014

NB: PTB+ is smear-positive pulmonary tuberculosis

As described above, in Papers I and II, cross-sectional study designs were based on the cohorts of all forms of TB cases (Paper I) and on cohorts of all smear-positive TB cases (Paper II) over 15 years. We included all public health institutions (73 health centres and one hospital) providing DOTS service, all forms of TB cases (Paper I) and all smear- positive TB cases (Paper II) diagnosed and registered for treatment between 1997 and 2011 in the study area.

A population-based cross-sectional survey with a multi-stage cluster sampling method was used to estimate the prevalence of PTB+ and BCTB cases at the baseline study (prevalence in Paper III). After that, a prospective follow-up study design was employed to estimate the incidence of the disease (incidence in Paper III). In Paper IV, the same study design and study population described in Paper III during the same study period were used to measure the burden of primary and secondary drug resistance, including MDR-TB in the study area. In the estimation of PTB+ and BCTB incidence, individuals free from symptoms suggestive of TB (SSPTB) at baseline survey, as well as those who had SSPTB at the baseline survey but who later showed negative bacteriological test results, were taken as a cohort for the prospective follow-up study. The study was carried out from July 2013 to June 2014.

Data collection procedure

We collected data from all forms of TB cases (Paper I) and all smear-positive pulmonary TB cases (Paper II) diagnosed and treated in all 74 DOTS-providing health facilities in the Arsi Zone from 1997-2011.

We adapted standard formats from the National TB Control Programme to collect data from TB Unit registers. The data collectors obtained information on the sex, age, address, TB type, patient category, date treatment started and HIV testing and their status from the registers (Paper I). In Paper II, in addition to data obtained in Paper I, information on the contact person for tracing, drug regimen, treatment follow-up, follow-up sputum smear microscopic result and treatment outcomes was collected. Moreover, the data collectors interviewed the head of each health centre on the availability of TB drugs, TB laboratory services, reagents and anti-TB drugs. We conducted the study from January to March of 2013 in all DOTS-providing health facilities (Papers I and II).

In Papers III and IV, house-to-house visits at the baseline survey conducted from May to June 2013, and the subsequent prospective follow-up from July 2013 to June 2014, were carried out to identify individuals with a persistent cough of more than two weeks, fever, loss of appetite, weight loss, blood-stained sputum and chest pain or difficulty of breathing, all of which were considered as symptoms suggestive of pulmonary TB. Study participants with such symptoms

were interviewed about their age, sex, educational status, history of TB contact and their current and previous TB treatment, and were requested to submit recently-discharged adequate mucoid or muco-purulent (spot-morning), one at the spot and the other on the following morning at their house. Immediately upon receipt from the presumptive, the specimens were put in sterile flacon tubes and placed in a cold box at 4^{o} and transported on the same day to the Adama Regional Research Centre Laboratory.

The sputum specimen was fixed, air-dried and stained using the standard Ziehl Neelsen methods (ZN) [199], and examined by experienced laboratory technicians for the presence of acid-fast bacilli (AFB) (Paper III). Positive results were quantified according to the International Union against Tuberculosis and Lung Disease (IUATLD) standards [222]. Moreover, the obtained morning specimen was digested and decontaminated by the standard Acetyl L-cysteine (NALC)-NaOH method [18], and inoculated into a Lowenstein-Jensen (LJ) culture medium for laboratory examination (Papers III and IV).

Drug susceptibility tests were carried out using the simplified indirect proportion method on a LJ medium. The proportion method validates the percentage of growth of distinct inoculums on a drug-free control medium compared to growth on culture media containing the critical concentration of anti-tuberculosis drugs. The resistant strains were determined using the percentage of colonies that grew on the critical concentration of 0.2 mg/l for Isoniazid, (INH), 4 g/l for Streptomycin (STM), 40 mg/l for Rifampicin (RIF) and 2 mg/l for Ethambutol (EMB). The isolate was said to be drug resistant when the growth was more than- or equal to 1% of the bacterial population on the media containing the critical concentration of each drug (Paper IV).

Definition of terms

TB Diagnosis, classification, case definitions and treatment outcomes were presented in each individual paper, with some important definitions described below:

Pulmonary TB smear-positive (PTB+) is a patient with at least two initial sputum smear-positives for acid-fast bacilli (AFB) by direct microscopy or a patient with only one sputum smear-positive for AFB, and with chest radiographic abnormalities consistent with active pulmonary TB followed by a clinician's decision.

Bacteriological-confirmed TB (BCTB) is defined as an individual with smear- and/or culture-positive results

Pulmonary TB smear-negative (PTB-) is a patient with at least three initial sputum smear-negative for AFB by direct microscopy, and with chest radiographic abnormalities consistent with active pulmonary TB and no clinical response to two weeks of broad spectrum antibiotic therapy followed by a clinician's decision.

Extra pulmonary tuberculosis (EPTB) is tuberculosis involving organs other than the lungs, such as the skin, abdomen, joints and bones, lymph nodes, pleura, genitourinary tract and meninges. The diagnosis is based on fine needle aspiration (FNA) for histopathological examination or biochemical analysis of ascetic/pleural/cerebrospinal fluid followed by a clinician's decision to treat it with a full course of anti-TB drugs.

New TB case not on treatment: TB patients who did not treat for more than one month in the past and who are not currently on anti-TB drug treatment.

New TB case on treatment: TB patient who is currently on anti-TB drug treatment, but who had never been treated with anti-TB drugs in the past.

Previously treated TB case not on treatment: TB patient who was treated for more than one month in the past, but not currently being treated with anti-TB drugs.

Previously treated TB case on treatment: TB patient who was treated for more than one month in the past and is currently on an anti-TB drug treatment.

Failure: TB patient who has been treated for the disease, but remains or becomes sputum smear-positive at the 5th month or later.

Cured: Pulmonary smear-positive TB patient whose sputum smear microscopy was converted to negative at the end of treatment, with at least a smear-negative result either at the 2nd or 5th month of treatment.

Treatment completed: Pulmonary smear-positive TB patient who completed the full course of TB treatment, but who does not have a sputum smear result at the end of treatment or does not fulfil the criteria to be categorized as failure, or EPTB and smear-negative patients who completed the full course of treatment.

Case detection rate: The number of pulmonary smear-positive TB patients detected among the total estimated cases in a given population expressed by percentage.

Treatment success rate: The number of TB cases cured or completed the treatment out of the total TB cases reported expressed as a percentage.

Relapse: The re-diagnosis of smear-positive TB cases who had been declared as being successfully treated.

Recurrence: The re-diagnosis of TB among patients who had been declared as being cured or who completed treatment in the past with or without a smear-positive result, including relapse cases.

TB Case notification: The number of TB cases notified from a particular district divided by the total population of the same district in a given year.

TB case detection rate: The number TB cases notified from a given population divided by the total number of expected TB cases from the same population in a given year multiplied by 100

Exposure variables and study outcome measures

The study outcome measures; TB case notifications of smear-positive, smear-negative and EPTB cases, in addition to the case detection rate of smear-positive TB cases (Paper I). In Paper II, the outcome measures were treatment outcome that included the proportions of treatment success (treatment completed or cured), proportions of lost-to-follow-up, deaths, relapses, treatment failures and transfers. In Paper III, the prevalence and incidence of bacteriologically confirmed pulmonary TB cases were the outcome measures, while primary and secondary resistance to any anti-TB drugs and multi-drug resistance TB (MDR-TB) were the outcome measures of Paper IV. Socio-demographic variables such as age, sex, place of residence and population ratio to number of DOTS sites in the districts (in Paper I), age, sex, place of residence, patient category, HIV status, contact person for tracing during lost-to-follow up (in Paper II) and sex, age, residence, level of education, history of TB treatment and history of TB contact (in Papers III and IV) were all used as exposure variables in the studies.

Sample size and sampling techniques

In Papers I and II, we enrolled all forms of TB cases (Paper I) and all smear-positive TB cases (Paper II) diagnosed and treated in all 74 public health institutions providing DOTS services between 1997 and 2011 in the Arsi Zone. As result, a total of 41,965 for all forms of TB cases (Paper I), and a total of 14,221 smear-positive pulmonary TB cases (Paper II) that were diagnosed and treated during the years mentioned, were all included in the studies.

In Papers III and IV, we used a stratified multi-stage random sampling procedure to select 49 clusters (*garees*) from all *kebeles* of the district that were taken as a larger primary sampling unit, and selected a *garee* from each *kebele* taken as a smaller secondary sampling unit of the study. The number of clusters allocated to each *kebele* was proportional to its population size. Based on the urban and rural population proportion, six clusters for urban and 43 clusters for rural *kebele*s were also allocated.

A cluster is defined as a *garee* within a *kebele,* with approximately 650 adults aged ≥15 years [223]. Alphabetically arranged, the list of *garees* in each *kebele,* along with their population size, was obtained from the district authorities. The *garees* were then randomly selected from the list and included in the study. All individuals aged ≥15 residing within the selected *garee* were included in the study.

The sample size for the study was calculated based on the prevalence of PTB+ estimated by the WHO for Ethiopia in 2012 (210/100,000 including children) [100]. However, the target population for the study included individuals aged ≥15 years, in which the proportion of population in this age group was estimated to be 55% of the total Ethiopian population [184]. Therefore, the prevalence of PTB+ of individuals aged ≥15 years was estimated to be 210/0.55 per 100,000 population (382 per 100,000).

Based on the assumption of a PTB+ prevalence of 382 per 100,000 among the adult population aged 15 years and above, a relative precision of 0.25, an expected participation rate of 95% and a design effect of 2 [224] of the study area, a sample size of 33,661 people from 49 clusters was calculated. However, in the house-to-house enumeration during the pre-survey of all the 49 selected *garee*s*,* 34,707 adults aged ≥15 years were identified, and all were included to improve the precision of the studies.

Data analysis and statistics

Data were coded and double entered by trained data clerks using Epi-info statistical software version 7 (Paper I-IV). We analysed the data using SPSS for windows version 20 (SPSS Inc. Chicago, IL) (Papers I, II and IV) and STATA (v12.1, STATA Corporation, USA) (Paper III). Descriptive analyses such as frequency, mean and standard deviation were computed as appropriate. Univariate and multivariate analyses were carried out and an Adjusted Odds Ratio (AOR) was used to compare groups to determine the strength of association between the study variables at a 95% confidence interval (CI) for all studies. Moreover, a Poisson regression analysis was carried out in the analysis of TB incidence (Paper III). The estimated incidence rate ratios (IRRs) for the incidence rate and AOR at a 95% CI for the TB prevalence were used to assess the strength of association with PTB+ and BCTB cases as an outcome.

In Paper I, the population size used as a denominator to calculate the TB case notification from the 25 districts of the study area was based on the National Censuses of 1997 and 2007 obtained from the Central Statistics Agency (CSA) [29, 30]. The mid-year population of each district for each of the 15 years was extrapolated from the two censuses, and the 15-year average population for each district was then obtained by adding the mid-year population for each of the 15 years (1997 to 2011) in each district and then dividing it by 15. The 15-year average of all forms of TB cases for each district was also computed by adding all forms of TB cases notified by each district in each year (between 1997 and 2011) and dividing it by 15. The same procedure was followed to compute the 15-year average of PTB+ case for each district. Finally, the 15-year average of all forms of TB case and PTB+ case notification for each district was computed by dividing the 15-year average of all forms of TB and PTB+ cases notified in each district by the 15-year-average mid-year population of their respective district, then multiplying it by population of 100,000.

Moreover, in Paper I, 104/100,000 smear-positive TB cases obtained from the 2011 National TB Prevalence Survey for Oromia (9) was used as the expected number of smear-positive TB cases (denominator) in the calculation of the PTB+ case detection rate (CDR) for each year (1997 to 2011). In this case, the PTB+ CDR was calculated by taking the total number of PTB+ cases notified in each year in the zone as the nominator and the total expected PTB+ incidence cases of the zone for each year as the denominator, and multiplying it by 100 to determine the trend over the years.

In the estimation of smear-positive pulmonary tuberculosis and bacteriologically confirmed TB (Paper III), a complete analysis was made using robust standard errors to account for the sample cluster survey design effect, whereas the adjusted estimate prevalence of PTB+ and BCTB were also computed and reported [225]. Moreover, in the analysis of TB incidence, a person-year observation was used as a denominator, in which a person-time at risk of a TB case began in June 2013 when eligible individuals started to participate in the study. The enrolment ended when the study participants were found to be AFB or culture-positive, or censored in June 2014.

Data quality assurance

Data quality assurance activities included the use of well-established laboratory procedures and definitions, the use of experienced and trained laboratory technologists and data collectors, and strict supervision. To ensure the validity and consistency of the instrument, a standard and pre-tested structured questionnaire was used (Papers III and IV), and standard formats adopted from the National TB Control Programme were utilized to collect the data from TB Unit registers (Papers I and II). The overall data collection process was coordinated and supervised by the principal investigator, and all smear-positive and 10% of smear-negative slides were re-examined by a senior laboratory technologist who was blinded to the first test results (Paper III). However, there was no discordance in both test results. Training was given to data collectors on the objectives of the study, the contents and administration of the questionnaire, and on how to collect and transport sputum from the field to the laboratory. All TB Unit registers used in each TB treatment unit during the study period were checked against the total number distributed from the zonal health office during the study period. Indeed, no missing TB Unit registers were detected during the study period (Papers I and II). Likewise, throughout the entire data collection process,

the data were checked by district health facilities and the year of treatment against unit TB registers for consistency and completeness.

The principal investigator also checked 5% of the completed questionnaires on a daily basis during data collection in the field for its completeness. Standard laboratory procedures were followed during the sample collection, transportation and examination, and all laboratory preparation and examination procedures were carried out by experienced laboratory technicians following the standard laboratory guidelines of the WHO.

Ethical considerations

The Regional Committee for Medical Research Ethics in Western Norway (REK Vest) and the Ethics Committee at the Oromia Regional Health Bureau in Ethiopia approved the study protocol. Household heads, presumptive TB cases and known TB cases under treatment at the time of the survey were interviewed after obtaining informed verbal consent. The information of each interviewee was kept confidential, and before the start of field data collection, discussions with both government officials and community leaders at various levels were made, and permission was obtained.

Main findings

Trends in TB case notification (Paper I)

A total of 41,965 TB patients were registered in the 25 districts of the Arsi Zone between 1997 and 2011. More than half of them (22,743 or 54.2%) were males. In terms of residence, 27,913 of the patients (66.5%) were from the rural areas. Additionally, of the total number, 39,010 (93%) were new, 15,370 (37.5 %) were PTB15, 102 (36%) were PTB- and 11,447 (27.3%) were EPTB tuberculosis cases.

The trend in TB case notification increased in parallel with the expansion of DOTS population coverage, and went up from 18% (5) to 70% (74) in 15 years (X^2 trend = 75.2, P < 0.001). Case notification increased from 14 to 150 for all forms, and from 7 to 63 for PTB+ per 100,000 population over 15 years, with an overall increase of 90% for all forms and 89% for PTB+. The trend in TB case notification steadily increased in the first four years of the DOTS introduction between 1997 and 2000. It rose from 14.3 to 96.5/100,000 population for all forms, and from 7 to 42/100,000 population for PTB+ TB cases. The trend stabilized between 91.2 and 104/100,000 population for all forms of TB, and between 37 and 42/100,000 population for PTB+ cases during the years between 2001 and 2006. The trend increased again to 128 for all forms and to 52 for PTB+ in 2007, and reached 150 for all forms and 63 for PTB+ per 100,000 population in 2011.

The TB case detection rate (CDR), estimated by the proportion of PTB+ cases, notified from the total annual expected PTB+ incidence cases of the zone, went up from 6.4% in 1997 to 34.5% in 2001, and from 48.3% in 2007 to 58.7% in 2011 (X2 trend = 26.8, P <0.001). The overall 15-year average of PTB+ case detection rate was 42.3%.

The 15-year average of TB case notification across the 25 districts of the zone was unevenly distributed. The highest, 636 for all forms and 163/100,000 population for PTB+ case notification, was from Assela Town, followed by 314 for all forms and 150/100,000 for PTB+ case notifications from the Dodota District. The highest (24.2%) TB-HIV co-infection was also observed in Assela Town, followed by 16.4% in the Dodota District, while the zonal average was

9.4%. Nevertheless, the lowest 15-year average of 63 for all forms and 11 for PTB+ per 100,000 population was observed in the Tiyo District, where the TB-HIV co-infection was 6.9%. This was lower than the zonal average, and also that of Assela Town and the Dodota District.

Rural residence (AOR, 0.23; 95% CI: 0.21 to 0.26) and districts with a population of more than 25,000 per DOTS site (AOR, 0.40; 95% CI: 0.35 to 0.46) were associated with a low TB case notification. However, TB case notifications were high among those between 15-24 years of age (AOR, 1.19; 95% CI: 1.03 to 1.38), PTB- (AOR, 1.46; 95% CI: 1.33 to 64) and EPTB (AOR, 1.49; 95% CI; 1.33 to 1.60) TB cases.

Trends in treatment outcomes among PTB+ (Paper II)

From a total of 15,370 PTB+ cases registered between 1997 and 2011 in the 25 districts of the Arsi Zone, 14,221 (92.5%) were evaluated. The treatment outcomes of 521 PTB+ patients had incomplete records and 628 transferred out, and hence with unknown treatment outcomes, were excluded from the analysis. From the total evaluated TB cases, 7,734 (54.4%) were males, whereas the remaining were females. Furthermore, 5,119 (34%) were urban residents, 13,237(93%) were new, 867 (6.1%) were relapse cases, 51 (0.4%) were failure and 66 (0.5%) were returnees after default.

Trends in TSR increased from 61.3% to 91.2%. This was in parallel with the increase in DOTS population service coverage, which rose from 18% in 1997 to 70% in 2011. The overall TSR increased by 30%, while DOTS service coverage rose by 52%. The TSR steadily increased during the years between 1997 and 2001, with the exception of 1999, and then stabilized in the range between 82.1% and 84.2% through the years between 2003 and 2008. It then increased to 91.7% in 2011.

The trend in death rate remained at a high rate, ranging between 12.5% and 8.8% from 1997 to 2005, followed by a gradual decline to 3.9% in 2011. The trend in default rate also declined steadily from 29.9% in 1997 to 2.1% in 2011 (X2 trend, 18.56, $p<0.001$).

From the 14,221 PTB+ cases evaluated, 11,888 (83.6%) were successfully treated and 9,608 (67.5%) were cured, whereas 2,333 (16.4%) were unsuccessfully treated. Of these, 1,048 (44.9%) died, 1,215 (52.1%) defaulted and 70 (3%) had treatment failure. The TSR among new PTB+ was high (84.8%) compared to the 67.5% among re-treatment cases. Among the latter cases, the death rate was 11.7%, the default rate was 19.2% and the rate of failure was 5.6%. Moreover, the death rate of those re-treated after default was 15.2%, while it was 15.7% for those re-treated after pervious failure and 11.2% for those after previous relapse. These figures are high when compared with death from new PTB+ cases (7%).

Treatment outcomes varied across the 25 districts of the zone (X2_317. 35, $p<0.001$). The 15-year average TSR varied from 69.3 to 92.5%, while for the defaulter rate, it ranged from 2.5 to 21.6%. During the same period, the case fatality rate ranged from 1.6 to 11.1%.

A multivariate logistic analysis showed that TB patients in the age group between 25-49 (AOR, 0.26; 95% CI: 0.53-0.95) and above 50 years of age (AOR, 0.42; 95% CI: 0.33-0.60) were less likely to be successfully treated compared to the younger age groups. Re-treatment cases (AOR, 0.61; 0.41, 0.67) compared to new cases, and TB-HIV co-infected cases (AOR, 0.45; 95% CI: 0.31-0.53) compared to non-TB-HIV co-infected cases, were also less likely to be successfully treated.

Incidence and prevalence of smear-positive TB (Paper III)

A total of 33,073 individuals were screened for symptoms suggestive of TB at the prevalence survey, and 32,800 were identified for the follow up study. Of the former, 27,173 (82%) were rural residents. Gender-wise, 16,907 (51.1%) of them were males, while the rest were females. At the baseline survey, 1,041 individuals, and during the follow-up study, 1,468 individuals, reported to have symptoms suggestive of TB. They therefore provided two sputum samples for bacteriological examination.

Examination results revealed that 43 out of the 1,041 presumptive TB cases were bacteriologically confirmed TB (culture- and/or smear-) positive at the baseline survey. From the 43 bacteriologically confirmed TB **(**BCTB), 36 were smear- and culture-positive, while seven were only culture-positive. From the 36 cases, 24 (66.7%) were new, whereas 12 (33.3%) had a previous history of TB treatment.

The overall adjusted weighted prevalence rate of PTB+ among adult individuals aged ≥15 years was 109 (95% CI: 73.3.-144), while that of BCTB was 132 (95%CI: 91.8-169) per 100,000 population. Although there was no statistically significant difference, the weighted prevalence of BCTB among females (149.3) (95%CI: 91.1-209.3) was higher than males (114.2) (95%CI: 64.0-165.0).

In the multivariate logistic regression model, age and the family history of TB contacts were independently associated with high rates of PTB+ and BCTB cases. Compared to individuals in the age group between 15-24 years, those in the age group between 25-34 years [AOR: 3.45 (95% CI: 1.38-8.61)], 35-44 years [AOR: 4.18 (95% CI: 1.71-10.20)] and those ≥45 years [AOR: 2.76(95%CI: 1.14-6.72)] were more likely to have TB. Furthermore, those with a history of contact with TB patients in the family were 13 times more likely to develop TB than those without such a history [OR = 13.0, (6.55-25.33)].

The active and passive TB case findings of the study area were compared using the number of TB cases identified using each method. Forty-three BCTB cases were identified through active TB case findings, while 45 PTB+ cases were detected through passive case findings. Of the 45 PTB+ cases, 28 were males and 17 were females, thus creating a ratio of 1:0.65 (28/17). The male-female ratio for those identified through an active case finding was 0.79:1 (19/24). The ratio of passive-to-active case findings was 1:0.96 (45/43), which means for every one TB case identified through a passive case finding, there was almost an equal number (0.96) of undiagnosed infectious TB cases in the community.

A total of 32,800 individuals were enrolled in the follow-up study. Of these, 32,759 were followed up for 12 months, comprising a total of 393,108 person-months, with 41 diagnosed with TB at the end of the sixth month. They carried out 246 person-months of observation, making a total of 393,354 person-months, or 32,779.5 person-years observed. However, there were 18 adult deaths, 47 refusals and 121 out-migrations. Additionally, 1,634 individuals who did not participate in the baseline survey were excluded from the follow-up study. From the 32,779.5 person-years observed, 76 were bacteriologically confirmed TB cases, and of these, 70 were smear- and culture-positive, while six were only culture-positive.

The incidence rate of PTB+ and BCTB among adult individuals aged ≥15 years was 213.5 (95%CI: 187.9-238.9) and 231.9 (95%CI: 205.2-258.2) per 100,000 person-years, respectively. The incidence of PTB+ cases was 214.9 (95%CI: 179.2-250.5) among males and 211.9 (95% CI: 175.6-248.2) among females per 100,000 person-years, whereas it was 219.8 (95%CI: 158.6-281.1) among urban dwellers and 210.6 (95%CI: 182.7-238.5) among rural residents.

In the multivariate Poisson regression analysis, the age and history of TB contact were independently associated with a high risk of TB. Presumptive TB cases in the age group between 35-44 years were 2.4 times [aIRR, 2.40 (95% CI: 1.15-5.0)] more likely to have TB compared to the younger age group between 15-24 years. Compared to the same age group, those with an age of ≥45 were 1.8 times [aIRR 1.80 (95%CI: 1.00-3.50)] more likely to develop the disease. Those with a history of contact with TB patients in the family were 5.11 times more likely to have TB than those with no history of such a contact [aIRR, 5.11(95%CI: 2.63-9.96)].

Primary and secondary MDR-TB (Paper IV)

Out of the total of 33,073 individuals screened for symptoms suggestive of PTB, 16,888 (51%) were males. Furthermore, 28,048 (84.8%) were rural residents, while the remaining 5,025 (15.2%) lived in urban centres. Of the screened individuals, 2,758 (8.3%) were reported to have symptoms suggestive of PTB. Of these, 2,218 (80.4%) were new cases, whereas 540 (19.6%) had treatment with first-line anti-TB drugs.

All of the 2,758 who reported to have symptoms suggestive of TB gave spot and morning sputum for culture examination, and 106 (3.8%) of them were found to be culture-positive for *Mycobacterium tuberculosis*. Twenty specimens were contaminated and excluded from the analysis. From the 106 culture-positives, 85 (80.2%) were new and 21 (19.8%) were previously treated TB cases. Of the total isolates, 83 (78.3%) were susceptible to all first-line anti-TB drugs, whereas 23 (21.7%) were resistant to one- or to a combination of first-line anti-TB drugs. Five cultures (4.7%, 95% CI: 2.8-6.6 %) were MDR-TB cases.

Of the 85 new *M. tuberculosis* isolates, 13 (15.3%) were found to be primary-resistant strains to any one or more of the first-line anti-TB drugs. Primary MDR-TB was detected in two (2.4%) strains. Of the 21 previously treated *M. tuberculosis* isolates, 11 (52.2%) were found to have developed a secondary resistance to one or more of the first-line anti-TB drugs. Secondary MDR-TB was detected in three (14.3%) isolates.

A previous history of TB treatment and urban residence was independently associated with a high risk of resistance to any first-line anti-TB drug. Individuals with a previous history of TB treatment were eight times (adjusted odd ratio (AOR), 8.1; 95% CI: 2.3-29.3) more likely to develop a resistance to any first-line anti-TB drug than those with no such history. Similarly, urban residents were four times (AOR, 4.1; 95%CI: 1.3-12.8) more likely to develop a resistance to any first-line anti-TB drug compared to their rural counterparts. Individuals with a previous history of TB treatment were nearly seven times more likely (AOR 7.1; 95%CI: 2.6-43.8) to have MDR-TB compared to those who had no history of previous exposure to anti-TB drugs.

Discussion

Methodological considerations

Study design

This study employed different designs, as the choice of specific epidemiological study design is determined by the research question under investigation, its feasibility, the cost and its time demands [226].

In this thesis, we used a cross-sectional study (Papers I and II) and a cross-sectional at baseline and prospective cohort study designs for Papers III and IV. In Papers I and II, we used cross-sectional- and a retrospective trend analysis based on the cohorts of all forms of TB cases (Paper I) and on cohorts of all smear-positive TB cases (Paper II) over 15 years. Such a study design could allow the investigator to examine the relationship between diseases and other variables of interest in a defined population at a given point in time or over a short period of time. As mentioned above, it is also used to determine the burden of a disease or the health needs of a population at a given point in time that can serve as input for planning and health resource allocation. However, in cross-sectional studies, the exposures and outcome of interests are measured simultaneously, so it is therefore difficult to determine whether the exposure preceded or followed the disease [227].

Retrospective trend analyses were employed in Papers I and II, which are efficient because they cost less and can be done in a relatively short time. Such designs are useful for unusual or rare exposures. Moreover, they are essential in describing the trends in TB case notifications and treatment outcomes over the years, as well as trends in DOTS expansion [155].

However, the strengths of such studies are determined by the quality and completeness of the data. Such registry studies have some limitations since we did not collect socio-economic and environmental data. The absence of such data, and the fact that we did not adjust for possible unknown confounding factors represents limitations in our study design.

In Paper I, there might be some inaccuracy in TB case notification of the districts due to the extrapolation of census data from the 1997 and 2007 reports, and this might have affected the denominator of the population for each district. Also, the population growth rate over the years of

the study period and in the districts is not evenly distributed. As a result, there could be either an underestimation or overestimation of TB case notification, but we believe this potential error is small.

Additionally, TB case notification in some districts could be overestimated due to the fact that they may have health facilities with a good history of TB care that could have attracted more patients from others districts. Even if this error could result in both over- and underestimations for each district, the case notification rates in the respective zone would not be affected [228].

In Paper I, approximately 0.9 % of the notified TB cases did not have a complete record, while in Paper II approximately 3.4% of them did not have complete treatment outcome records. However, as the number of cases with incomplete records was relatively small, this might not affect the overall findings of the studies. Nonetheless, in order to increase the validity of the result, all patients who were treated with a full course of anti-TB drugs, but with missing records on their treatment outcomes, should be considered as defaulters. Even so, we excluded these cases from the analysis and an intention to treat was not used during the analysis. The exclusion of such missing records in this study could underestimate the defaulter rate, and subsequently undermine the unsuccessful treatment outcomes but overestimate the successful treatment outcomes.

A cohort study is defined as a study design where a cohort of the population is followed over a period of time to determine the association between risk factors and the development of an outcome of interest. Cohort studies can be classified as prospective, retrospective or a combination of both depending on when the outcomes of interest occurred in relation to the enrolment of the cohort.

In a prospective cohort study, the investigators identified the study population at the beginning, collected baseline data and followed it up over a period of time in order to determine the development of an outcome of interest. In contrast, retrospective cohort studies were considered after some members of the population already developed the outcomes of interest, in which the investigators had to go back in time to identify the exposure.

In this thesis, studies on the prevalence and incidence of smear-positive TB (Paper III) and primary and secondary anti-TB drugs resistant to TB (Paper IV) employed a combination of cross-sectional- and prospective cohort study designs. A population-based cross-sectional survey was used to estimate the prevalence of smear-positive TB. and anti-TB drug resistant TB at baseline. Next, a prospective cohort study was used to estimate the incidence of smear-positive TB (Paper III). Additionally, a combination of cross sectional study design at baseline survey and prospective cohort study design to determine the burden of anti-TB drug resistance (Paper IV).

As mentioned earlier, cross-sectional studies employed in the baseline survey are useful for priority setting in resource allocation and public health planning, as they provide valuable information on disease burden, health status and the health-care needs of a population. The findings might also lead to generating a testable epidemiological hypothesis, and would therefore enable the evaluation of the performance of the DOTS strategy in the TB control programme. However, as described above, such a study design can only be applied at a point in time, and does not give the sequence of events. In other words, it does not show whether the exposure occurred before, during or after the onset of the disease.

In the prospective follow-up studies (Papers III and IV), individuals who were free from symptoms suggestive of TB (SSPTB) at baseline, and those with a negative laboratory test result for TB, were enrolled in the prospective cohort. The findings of the studies might estimate the incidence of TB [210]. The result could also help to measure the impact of TB control efforts in the country [201, 212].

Nevertheless, a prospective cohort design has different limitations, such as a long observation time until the outcomes of interest develop, high costs, inappropriateness for rare diseases, and diseases with a long latency and possibility of differential affecting lost to follow-up. However, those lost to follow-up in our studies were small [185 out of 32,800 (0.6 %)], and probably did not affect the results.

Selection and sample size

The sample size calculation for Papers I and II was done using OpenEpi.140 and a population size (N) of 3,100,000 in the Arsi Zone. The hypothesized proportion of the outcome measure of case detection or treatment success at 50%, 95% CI had a margin of error = 5%, with a design effect of 2. The calculated sample size for Papers I and II would be 1,202. However, all forms of TB cases reported as being diagnosed and treated during 1997-2011 were collected from all health facilities and included in the study. As a result, a total of 41,965 TB patients diagnosed and treated in all health facilities (74 health facilities in total) providing DOTS services during the study period in the Arsi Zone were included. TB Unit Registers in all health facilities during the study period were identified by the principal investigator and brought to the regional health bureau office between January-March 2013. Subsequently, TB patients' information on sex, age, address, TB type, patient category, date of treatment started, and HIV testing and their status from the TB Unit Registers, were collected and entered onto a computer programme (SPSS version 20).

In Papers III and IV, we used a stratified multi-stage random sampling procedure to select 49 clusters (*garee*s) from all *kebeles* of the district. All *kebeles* in the district were primary sampling units and the *garees* from each *kebele* were the smaller and secondary sampling units. A cluster was defined as a *garee* within a *kebele,* where roughly 650 adults of age ≥15 years were residing [223]. Clusters (*garees*) from each *kebele* were also randomly selected and included in the study.

We calculated the sample size of the study on the basis of the prevalence of PTB+ estimated by the WHO for the adult population in Ethiopia [100], and by using the assumption in the national TB prevalence survey [229]. Based on the estimation, a relative precision of 0.25, an expected participation rate of 95%, a design effect of 2 and a sample size of 33,448 people from 49 clusters was calculated. However, in the house-to-house enumeration during the pre-survey of all the 49 selected *garees*, 34,707 adults aged ≥15 years were identified. To help increase the reliability of the study, we included all adult individuals identified during the house-to-house enumeration. Moreover, the estimated design effect we used to calculate the sample size of the study was 2, but the actual calculated design effect from the study was 1.3, thereby implying that the sample size for Papers III and IV was adequate.

Validity of the studies

The findings of any research can be affected by the quality of the data used, which in turn can be affected by the quality of the instruments used to collect the data, biological variation and respondents' memories. This means that any epidemiological study cannot be free of errors, but efforts should be made to minimize them [230]. Therefore, the validity of such a study is determined by the choice of study design, the procedure used to conduct the study and the data analysis [231]. The validity of a study refers to the accuracy with which the study measures its findings. In terms of population, validity is divided into internal validity and external validity.

Internal validity

Internal validity is the accuracy in measuring what the study has intended to measure in the participants or refers to the extent to which alternative explanations such as chance, bias or confounding variables accounted for the observed association [226]. Whenever the findings of an epidemiological research reflect the real effect of exposures on the development of the outcome of interest, the results may, in fact, be due to the effect of an alternative explanation [226].

These alternative explanations could result from the effects of chance (random error), bias or confounding, which may yield erroneous results that lead to the conclusion of the existence of a valid statistical association while there is no true association. Observational studies are primarily susceptible to the effects of chance, bias and confounding. Therefore, one should make an effort to minimize the effect of such alternative explanations, both at the design and analysis stages of any epidemiological study [231]. Such possible alternative explanations for the study findings are briefly discussed below.

Chance

Chance is a random error that may cause an association between an exposure and an outcome. The primary assumption in epidemiology is that it is possible to draw an inference about the experience of the entire population based on the evaluation of a sample taken from that particular population. Nonetheless, the problem with drawing such an inference is that chance can affect the results of the study due to the effects of random variation among samples [231]. For this reason, the effect of random error could yield an estimate different from that of the true underlying value, hence leading to either an over- or underestimation of the value.

In any epidemiological study, unless the entire population is studied, it is not possible to eliminate sampling errors, but reduce it to an acceptable level by increasing the sample size and using an appropriate study design [232]. As a result, studies in this thesis used a sufficiently large sample size and appropriate study design, thus keeping the variability due to chance to the minimum possible.

In fact, using a sufficiently large sample size and appropriate study design cannot completely eliminate random error from any epidemiological study. However, the error could be reduced and precision improved by increasing the sample size. Therefore, the role of chance in an epidemiological study needs to be assessed by performing an appropriate statistical test and calculating confidence intervals. Confidence intervals indicate if the association lacks precision as a result of a too small sample size.

In this thesis, we addressed the role of chance by performing appropriate statistical tests in estimating the confidence intervals. For instance, in the prevalence and incidence study (Paper III), the 149/100,000 bacteriologically confirmed TB prevalence among females was high compared to the 112/100,000 prevalence among males (AOR: 1.3 (0.71-2.43). Furthermore, the 274/100,000 person-years bacteriologically confirmed TB incidence among urban dwellers was high compared to the 223/100,000 person-years among rural residents (aIRR: 1.25 (0.72-2.16)). In the primary and secondary anti-TB resistance study (Paper IV), MDR-TB among urban residents was high when seen against its incidence among rural residents. However, these estimates lack precision, since they take 95% confidence intervals as an adjusted odds ratio and the incidence rate ratio crosses a null value. Consequently, we cannot completely exclude chance as a likely explanation for the identified result

Selection bias (systematic sampling error)

Selection bias is a systematic error in epidemiological studies that arises from the procedures used to select participants and from factors that stimulate study participation. It occurs when the association between an exposure and a disease varies for those participants and those who do not participate in the study. Selection bias results when study participants are inappropriately selected using different criteria or selected upon previous knowledge of exposures or outcomes. It can also result from voluntarily self-selection, the ascertainment of exposures or outcomes as a result of pervious knowledge, a one-sided loss to follow-up and a low response rate. In contrast to random error, selection bias cannot be reduced through an increased sample size. Hence, selection bias can only be managed at the design stage using an appropriate study design, and conducting the study using a clearly defined eligibility criteria [231].

There might be a bias in Paper I, as the study was based on a passive case notification of patients who may have access to health care, have an awareness of the availability of services and information on- or about the recognition of symptoms of the disease and have better health-seeking behaviour. However, patients with a lack of such access and information might be missed and remain undiagnosed in the community. Therefore, the cases occurring in the community may remain undiagnosed and would consequently underestimate the case notification of the disease.

For instance, we identified 109/100,000 PTB+ through active case findings in Paper III, whereas the 15-year average PTB+ case notification was only 42.3/100,000 in the study area, which suggested the underestimated TB case notification in Paper I. The diagnosis of PTB- and EPTB in the study area was based on smear microscopy and X-rays, followed by clinician decision due to the poor access to standard diagnosis facilities, such as culture and histopathology [233]. Thus, there might be a bias to over- or underestimate the PTB- and EPTB case notification in Paper I.

There may be a possible bias in Paper II because approximately 3.4% of the cohort did not have information on treatment outcomes. Patients with missing treatment outcomes might be different from those for whom a treatment outcome was recorded, which could have affected the results of the study. Still, the analysis of their baseline demographics, social characteristics and treatment regimen did not show any differences between the groups.

In Papers III and IV, as all adult individuals ≥15 years were selected from all *kebele*s of the district and included in the study, there is less likely a selection bias. However, because we excluded individuals who did not participate in the baseline survey, who refused to participate and out-migrants from the subsequent follow-up study, a bias due to the lost to follow-up of participants is unavoidable.

Information bias (misclassification)

Information or observation bias is a systematic error in a study that can arise when information is differentially collected from different study groups. Information bias can also be referred to as misclassification if the variable measured is categorical and the participant selected is placed in an incorrect category. Moreover, information bias can result from interviewer bias, recall bias, and can be also due to differential misclassification in the ascertainment of exposure or disease.

In Paper I, all TB patients registered for treatment over 15 years were included. All TB Unit registrars that had been distributed over those years from the zonal health office to all 74 TB treatment units of the zone were also checked. However, we did not find any mismatch between the numbers distributed and those available in the health facilities; in this case, the result of the study was less likely to be affected by systematic error. However, since as we used aggregate data for districts to compare TB case notification across districts in this paper, it could lead to an ecological fallacy.

Interviewer bias could affect studies in Papers III and IV, which employed interview questionnaires to obtain information on the potential risk factors and symptoms of suggestive TB. Furthermore, due to the stigma associated with TB, individuals with a previous history of the disease may not disclose their prior status, and may be registered as new cases (Papers I-IV). As a result, these patients may have been misclassified as new TB cases, thereby resulting in differential misclassification, which can impact the effect of a previous history of TB, secondary anti-TB drug resistance (Paper IV) and the direction of the observed association.

However, for Papers III and IV, we checked the TB patient registration units in the study area to verify whether those who reported that they were on anti-TB treatments at the time of the study were actually on medication, or if there was any patient who was on anti-TB medication but did not report so during the survey time. Still, no mismatch was observed, so misclassification was less likely to affect the result of these studies.

For the estimation of prevalence and the incidence of smear-positive TB (Paper III) and primary and secondary anti-TB drug resistance (Paper IV), Lowenstein Jensen (LJ) was used to culture the MTB. Culture is considered to be the gold standard for the diagnosis of TB [234]. We used two sputum samples collected, one on spot and the other in the morning, using experienced laboratory professionals. The collected samples were transported using the appropriate cold chain. However, the possibility of losing the viability of the bacteria cannot be fully excluded due to the long distance transportation of the samples. Moreover, we excluded 20 contaminated sputum cultures from the analysis at the baseline survey, which may have also affected the prevalence of sputum culture TB.

In Papers III and IV, symptoms suggestive of TB were used as a screening mechanism. The fact that a chest x-ray was not used in our study might have affected the prevalence, incidence and primary and secondary anti-TB resistances of TB in the area. We also carried out surveys three times: first, at the beginning of the study to determine the prevalence of TB, second, after six months, and third, at the end of 12 months to estimate the TB incidence. However, the six-month interval between the surveys may be a sufficient amount of time for a spontaneous self-cure of the disease, which might have led to an underestimation of the incidence of TB.

However, to maintain the quality of the study, rigorous training was given to data collectors and laboratory technicians. The study population was monitored to help identify deaths and migrations during the prospective follow-up study to provide an accurate time contribution in the denominator to compute the incidence rate.

Additionally, we used sensitive standardized and pre-tested questionnaires to screen presumptive TB cases, while experienced and qualified laboratory technicians carried out a smear microscopy and sputum culture. Following that a senior laboratory technologist, who was blinded for the first test results re-examined the entire smear-positive- and 10% of the smear-negative slides to help validate the quality of the laboratory results. We believe that the efforts we made to maintain the quality of the studies would minimize information bias, and hence improve the validity of these studies. Thus, these community-based TB incidence and drug resistance studies might be the first attempt in Ethiopia and other resource-constrained countries, and may help in generating hypotheses for further studies.

Confounding

This refers to mixed effects that can occur when the outcome measures of a study are confused by the effects of the third factor which should have an association with both the exposure and outcome, but is not an intermediary between them. A confounding variable can lead to an overestimation, underestimation or change in the direction of the observed association, and must always be accounted for. In contrast to random error, increasing the size of the study cannot affect the result of a confounding variable. Still, it can only be reduced at a design stage through randomization, restriction and matching, and at an analysis stage using stratification and multivariable modelling [230, 231, 235].

In the analysis, we stratified data by area of residence, age, type of TB and population ratio to the number of DOTS sites in the district (Paper I), area of residence, sex, age, patient categories and HIV status (Paper II), area of residence, age, sex, level of education and history of pervious TB (Papers III and IV); a multiple logistic regression (Papers I-IV) was also carried out to control for confounders. However, since all relevant variables that may determine the outcomes of interest were not included, stratified and analysed in these studies, we cannot fully claim that we have controlled all possible confounding variables.

External validity

External validity refers to whether the findings of an epidemiological study can be generalized to other populations. This study was conducted in urban and rural settings of the Arsi Zone of central Ethiopia, while similar findings were reported from southern Ethiopia [228, 233], which can typically represent urban and rural Ethiopia. However, variations in TB prevalence, incidence and anti-TB drug resistance (Papers III and IV) and TB programme performance (Papers I and II) in the country limit the generalizability of the results. Likewise, except for Paper I, all others focused only on smear-positive pulmonary TB, so the results of the studies cannot be generalized to other forms of TB. Moreover, with the exception of Papers I and II, studies in this thesis only included the adult population of ≥15 years. Therefore, the findings cannot be generalized to the population aged less than 15 years.

Main discussion

The success of a TB control programme is determined by its ability to detect all infectious TB cases and successfully treat them as early as possible, reduce transmission and prevent the emergence of drug resistance. The globally recommended DOTS strategy is intended to attain these goals [236]. In this study, it was found that expanding DOTS and improving its population coverage increased TB case notification and treatment success. On the contrary, there was a decline in the death of patients and loss of follow-up cases over the years.

However, we identified a variation in TB case notification and treatment outcomes across the 25 districts of the study zone. We also detected a high prevalence and incidence of smear-positive TB cases, as well as a high burden of primary and secondary drug resistance and MDR-TB. These findings are matters of great concern that warrant an alternative strategy to help the challenges. Thus, we believe that the findings of this study could help in improving the TB control programme in Ethiopia by informing the programmer managers and policy makers to devise an alternative and area-specific strategy so as to address the disparity in DOTS performance across different geographical settings and sub-optimal performance of DOTS in controlling disease transmission in the country.

DOTS was initially introduced in 1992 as a national pilot project in a health centre and a hospital in the Arsi Zone [199, 221]. It was then followed by a systematic scaling up of the control programme to other health facilities. This stepwise DOTS expansion began with health facilities found at the district capital, and then subsequently to sub-district levels. The coverage of all health facilities in the zone has been achieved in eight years. Furthermore, the continuous expansion in the zone over the past 15 years has been mainly due to a high political commitment in securing the necessary resources for the establishment of a laboratory network, treatment and monitoring, and also ensuring an uninterrupted supply of anti-TB drugs [172, 237].

In the years between 1997 and 2011, TB case notification increased from 14 to 150 for all forms and from 6.9 to 63 per 100,000 population for PTB+. Even so, this trend did not persistently continue throughout the study period. The overall 15-year average TB case notification of all forms of TB and PTB+ was very low compared to the expected global target. This might warrant the involvement of health extension workers in active TB case findings to help achieve the Sustainable Development Goals (SDGs) of a reduction in TB deaths by 90%, the TB incidence rate by 80% and zero TB-affected families facing catastrophic costs due to TB by 2030 compared to 2015 [86, 238].

The case notification rate (CNR) for all forms of- and smear-positive TB increased by nearly seven-fold and five-fold between 1997 and 2001, respectively. However, despite a notable increase in the number of TB diagnostic centres, CNR and the case detection rate (CDR) seemed to be stable during the years between 2002–2010. The most likely explanation for the increase in TB case notification during the first five years of the DOTS introduction could be due to the improvement in the recording and reporting of detected cases without a real increase in the detection rate [239]. Nevertheless, the increased trend could also be due to a true increase in TB incidence fuelled by the interaction between HIV and tuberculosis [70]. TB and HIV co-infection among tested TB patients in our study was 9.4%. It might also be due to the notification of the accumulated TB cases that remained in the community, which resulted from improved TB diagnostic access [239].

In the mathematical model used to predict the WHO target of a 70% TB case detection rate and an 85% cure rate in countries where the incidence of tuberculosis is stable and HIV-1 absent, there would be a reduction in the TB incidence rate by 11% and the death rate due to TB by 12% per year [240]. The stabilized CNR and CDR after 2002 might be due to an increase in case detection during the first five years, which could be offset by the decrease in the incidence of active TB. However, in this study, the 15-year average PTB+ CDR was low (37.7 %) and TB-HIV co-infection was high (9.4%). Consequently, this could not be the reason for the decline in TB incidence, thereby resulting in a stability in PTB+ case detection rate after six years of DOTS implementation. This indicates that the decentralization of TB services after certain points did not improve access to DOTS services as expected, which was primarily due to the insufficient decentralization of diagnostic and treatment services, inadequate health service coverage and a

shortage of health workers and resources. Thus, the expansion of DOTS sites may help to improve case notification to a certain point, while an increase in coverage may contribute to a marginal increment in case findings unless other community-level interventions are introduced [241].

In this study, we identified variations in PTB+ case notifications across the 25 districts with a 14-fold variation between different districts (10.9 to 150 in rural and 166 in urban areas per 100,000 population). The 15-year zonal average was 42.3 per 100,000 population. This variation in PTB+ case notification among districts may be an indication of an inequity in TB case findings or a heterogeneity in TB incidences across the 25 districts [203, 241-243], caused by a diversity in TB risk factors [237, 243]. For instance, the high 15-year average in PTB+ case notification (150 in rural and 166 in urban per 100,000 population) was observed among districts with a high TB-HIV co-infection. The difference in TB case notification could also be due to the defects in facility-based passive TB case findings of the DOTS policy applied uniformly to all districts with a different health service coverage, a different ability of the health system to detect TB cases and poor health-care-seeking behaviour of those with TB [244].

In this study, factors such as area of residence, age of patients, type of TB and the ratio of population size to DOTS sites were found to be associated with the level of TB case notification. This is in agreement with studies conducted elsewhere [245-247], in which TB case notification was associated with urban residence, age of patient, access to TB care and type of TB.

However, the study was based on retrospective facility-based data, in which some socio-economic and environmental data were not available and not included in the analysis. Therefore, the absence of such socio-economic and environmental data in the study could affect the result. There could also be a bias in TB case notification of the districts due to the extrapolation and use of 1997 and 2007 census data as a denominator due to the fact that there might be an unevenly distributed population growth rate across the districts. Moreover, comparing the aggregate TB case notification at a district level could lead to ecological fallacy.

In the meantime, there was an encouraging progress towards the WHO's intended target of 85% treatment success for new smear-positive TB cases. The treatment success rate (TSR) increased significantly from 61.3% to 91.2%, with a parallel expansion of DOTS population coverage that grew from 18% to 70% in 15 years. This upward trend in TSR was inversely proportional to the declining trend in defaulters, which was reduced from 29.9% to 2.1%, and deaths from 12.5% to 5.4%, over the study period. The increase in TSR between 2009 and 2011 was significant, and higher than the 85% recommended target by WHO. This steady improvement in treatment outcome, along with DOTS services expansion, confirms results by other studies [172, 239, 248] that DOTS works well in resource-constrained settings. The decentralization of DOTS services to community-based intervention has improved access to care and patient follow-up, which in turn increased treatment success rate.

The increase in TSR mentioned above was mainly due to the stepwise deployment of HEWs at the community level, which resulted in the improvement of community-based intervention [205] that improved access to TB control, together with an increase in primary health-care facilities and DOTS services [249]. The highest treatment success rate reported in 2011 mostly followed the change in treatment regimen of the continuation phase from ethambutol and isoniazid to rifampicin and isoniazid. This implies that rifampicin and isoniazid-based treatment in the continuation phase has decreased mortality during treatment, improved patient adherence and shortened the period of treatment from eight months to six months, in addition to reduced drug side-effects compared to treatment by ethambutol and isoniazid [250, 251, 252].

In this study, we found that patients returning for re-treatment after defaulting were much more likely to default again (26.6%) compared to new patients (7.0%). This finding is in line with previous reports from other studies [248, 253]. Poor adherence to anti-TB treatment due to defaulting and irregular treatment may lead to a more severe illness, treatment failure, relapse, longer infection, drug resistance, and even death. For this reason, defaulting and an irregular intake of anti-TB drugs are a challenge and concern for the individual patient, as well as for the community; as a result, they need to be addressed properly.

In our study, we identified a significant variation in treatment outcomes among patients across the 25 districts. The 15-year average TSR across the 25 districts of the zone ranged from 69.3% to 92.5%. TSR, in fact, was inversely proportional to the proportion of lost follow-up and failure cases. This indicates the role of effective TB treatment in the reduction of default cases and drug-resistant strains. The lowest TSR resulted from high failure and default cases, so death rates might reflect the consequences of poor TB treatment [118, 119]. Moreover, the high death rate observed among districts with a high TB-HIV co-infection in this study substantiates previous reports of a high mortality rate among TB-HIV co-infected cases [118].

In general, the variation in treatment success, default, death and failure rates across districts of the zone could be due to the real differences in DOTS performance and disparity in the quality of TB control programmes [248]. This warrants that TB programme managers and policy makers should identify locality-specific challenges to be addressed in order to universally achieve the global WHO recommended rate of an 85% treatment success.

We found out that the re-treatment of TB cases was significantly associated with unsuccessful treatment outcomes [248, 252-254], mainly due to a high death and failure rate among such cases compared to the new ones. The WHO recommends a microbial culture and drug susceptibility test (DST) for all re-treatment TB cases and new PTB+ cases that fail to convert sputum examination results at the end of the second month of follow-up [255].

Nonetheless, due to a limited access to culture and DST services in Ethiopia, the tests were not provided for MDR suspected cases; hence, the extent of MDR among re-treatment cases was not known. Furthermore, the high death and failure rate in this group in the current study might also be due to a high prevalence of MDR-TB in the group [252, 255]. The findings of this study warrant further investigation to determine the burden of MDR-TB in Ethiopia, and respond to the current global challenge of MDR-TB. Therefore, we conducted a population-based cross-sectional study to estimate the prevalence of primary and secondary multi-drug resistance to any first-line anti-TB drug and MDR-TB in the Hetosa District of the Arsi Zone.

Findings revealed that 15% of the newly diagnosed, and 52% of the previously treated TB cases, were resistant to one or more of the first-line anti-TB drugs. MDR-TB among new cases was 2.4% and 14.3%, respectively, among previously treated patients. Although the DOTS strategy with short course treatment regimens can achieve more than a 95% cure rate in newly diagnosed TB cases, drug resistance became the main cause of death and treatment failure, mostly when the strains are resistant to the two key drugs, isoniazid bactericidal and rifampicin [159].

Strains resistant to any one or more of the first-line anti-TB drugs and MDR-TB can be developed either when a person is infected with a resistant strain or when insufficient or improper treatment leads to drug selection of the resistant strain [159]. Strains with primary resistance to any of the first-line anti-TB drugs and primary MDR-TB can be developed when individuals with no history of first-line anti-TB treatment acquire the disease. When a person with no a history of first-line anti-TB treatment acquires resistance to any of the first-line anti-TB drugs, it is called a primary resistance to any first line anti-TB drugs and a resistance to rifampicin and isoniazid primary MDR-TB, but if the resistance is developed among previously treated TB cases, it is called a secondary resistance to first-line anti-TB drugs and MDR-TB, respectively [162].

In this study, we found a 15% primary and 52% secondary resistance to any one or more first-line anti-TB drugs, and a 2.4% primary and 14.3% secondary MDR-TB in the study area. As already discussed, previously treated TB cases were more likely to develop resistance to any of the first-line anti-TB drugs and MDR-TB compared to the newly diagnosed ones. Reports from elsewhere revealed that resistance to any first-line anti-TB and MDR-TB was more than 10-fold among previously treated TB patients than untreated cases [252]. A meta-analysis in Sub-Saharan Africa and a systematic review in Europe have also demonstrated a high pooled risk of MDR-TB among the previously treated TB cases compared to the new ones [256, 257].

The high prevalence of secondary drug resistance identified among previously treated TB cases compared to primary resistance among new TB cases in the current study can be ascribed to poor treatment outcomes among previously treated cases, which is caused by lost follow-up and an irregularity of drug intake [155]. Moreover, sub-optimal DOTS performance as a consequence of poor access to health facilities, unfriendly service providers, an irregular supply of drugs leading to monotherapy, poor patient orientation about the duration of the treatment and stopping

treatment when they feel better might have resulted in a secondary drug resistance [155], which might warrant further investigation.

The DOTS strategy aims to detect 70% of infectious TB cases and achieve a cure rate of 85% to interrupt transmission, reduce mortality and avert the emergence of drug resistance [14, 172]. However, reports from previous studies conducted in Ethiopia between 1984 and 2015 showed that the proportion of MDR-TB among new and previously treated TB cases varied from place to place and increased over time [258-260]. For instance, over 13 years, it increased from 0% to 2.4% among new patients and from 0% to 14.3 % among previously treated ones in the Arsi Zone [261].

Additionally, the 15-year average PTB+ case detection and cure rates in the study area were as low as 37.7 % and 66.9%, respectively - very far from the global DOTS strategy target. This, and the increasing trend in resistance to any TB drug and MDR-TB over time with the low TB case notification and cure rate in the study area could be an indication of a sub-optimal performance of DOTS in the area.

DOTS and DOTS-plus (addressing issues related to TB/HIV, MDR-TB, and the needs of poor and vulnerable populations) are believed to be an appropriate strategies to prevent the emergence of MDR-TB as they aim at accessing presumptive MDR-TB cases to diagnosis and appropriate treatment. Still, MDR-TB patients are challenged by a much more toxic and complicated treatment of a longer duration, which results in poor treatment outcomes and the emergence of XDR-TB. Therefore, the effective implementation of DOTS and DOTS-plus strategies is a cornerstone in the prevention of the emergence and spread of MDR-TB and XDR-TB in the country.

Thus, the emergence of drug resistance can be prevented through the use of proper treatment regimens taken at the right time, in the right dosage and for the right duration. This can only be ensured by the appropriate implantation of the DOTS programme [155]. Moreover, expanding MDR-TB diagnostic facilities and intensifying active case findings using health extension workers are urgent issues to be addressed in order to effectively control the increasing trend in drug-resistant TB in Ethiopia [205].

This is the first population-based study that analysed the prevalence of primary and secondary drug-resistant and MDR-TB in Ethiopia. It might also be one of the very few studies conducted in a poor resource setting. Thus, the finding of the study highlighted the real burden of anti-TB drug resistance at the community level. However, we only used symptoms suggestive of TB as a screening mechanism. The fact that a chest x-ray was not used could result in missing asymptomatic TB cases, thereby underestimating the burden of the disease. Moreover, there might be a misclassification of primary and secondary resistance to any one or more first-line anti-TB drugs and MDR-TB because the patients themselves may deny, or may not know that they have had a previous treatment for TB.

Furthermore, the sub-optimal DOTS performance in the study area warrants an investigation on the prevalence and incidence of TB in the area. The burden of TB in a population can be measured using various methods, as each method has its own strengths and limitations. In rich countries where there is a well-developed health-care delivery system, most of the active TB cases can be detected and an accurate estimate of TB incidence can be made. Nonetheless, health facility-based TB case notification in poor resource settings usually lacks completeness and consistency due to poor access to health facilities, an insufficient diagnostic network and a weak system of disease notification. In such settings, the notification cannot indicate the real burden of the disease within the community.

As an alternative, tuberculin surveys to determine the annual risk of infection (ARI) and a population–based survey using mass miniature radiography (MMR), followed by sputum microscopy, are used to estimate the prevalence and incidence of TB [262-264]. However, both methods are rarely used in poor resource settings because of constraints in finance and expiates. Hence, we conducted a population-based study to estimate the prevalence and incidence of smear-positive and bacteriologically confirmed TB cases in a predominantly rural district using symptom inquiry followed by a sputum microscopy for AFB.

In this study, for every PTB+ case receiving treatment during the survey, there was almost an equal number (0.96) of undiagnosed BCTB cases in the community. The findings further showed that there was a high proportion of undiagnosed infectious cases of TB. The undiagnosed TB cases in this study are very high compared to those reported by previous studies, in which it was revealed that for every undiagnosed infectious TB case, there were 2 to 2.5 PTB+ cases [265] in Ethiopia and 4.5 in South Africa [266] receiving treatment at the time of the study.

The high proportion of undetected infectious TB cases in the community might be due to the sub-optimal performance of DOTS, as indicated by a 15-year average of 37.7% PTB+ case detection and 66.9% cure rates, which are very low when compared to the 70% global target of the PTB+ case detection rate and 85% cure rate [14, 267, 268]. Moreover, the difference in the number of undetected infectious TB cases in the community across various geographic settings might be attributed to the variation in DOTS performance, DOTS service coverage and the quality of DOTS services across different study areas.

What is more, we identified a high prevalence of BCTB cases among younger age groups. This is in agreement with a previous report [196], and may suggest an ongoing transmission of TB in the community. Regardless of this, the high prevalence in TB incidence has shifted to an older age group. The high number of infectious TB cases identified through active case findings at baseline might reduce TB transmission in the general population, while the high TB incidence among the elderly may suggest a reactivation of latent TB among members of the group [196, 202, 253, 265].Even so, further study is required to fully understand why the high TB prevalence observed among the young has shifted to the older age group.

In this study, more men were on treatment before the survey, whereas more women were detected during the active TB case finding, which is in agreement with other reports in the country [265] and elsewhere in Asia and Africa [269]. Besides the possibility of real gender differences in TB epidemiology [270, 271], this finding indicates the possible existence of gender differentials in access to health care, in which women have less access and lower health-seeking behaviour [272, 273].

Moreover, a history of TB contact was found to increase the risk of developing active TB. This finding is in line with systematic reviews and large epidemiological surveys that have established an association between a history of TB contact and a higher risk of the disease [72, 246, 274]. Therefore, contact-tracing efforts should target household members diagnosed with PTB+, so as to better capture the undetected infectious TB cases within the community, thereby breaking the chain of transmission of the disease among household members in the family.

The high prevalence of BCTB cases identified in urban area in this study conforms to previous reports of a high TB prevalence in urban settings [204, 275, 276]. In contrast, the national TB survey reported a higher prevalence among rural residents [196]. This is due to the inclusion in the national prevalence survey of pastoralists in the rural population, in which the highest prevalence was observed among pastoralists at a ratio of 170/100,000 in the national survey [229], as opposed to the current study. The pastoralist population may have a poor access to TB care, as well as a low awareness and health-seeking behaviour, which might have resulted in them having a high burden of undiagnosed TB cases and eventually elevating the prevalence of TB among the rural population in the national prevalence survey. Moreover, the higher prevalence of BCTB cases among urban settings compared to rural areas in this study may be due to the presence of predisposing factors, such as overcrowded living conditions and a higher HIV prevalence than in the latter setting [181].

The high incidence of TB in the present study has confirmed previous reports from southern Ethiopia, South Africa and Guinea-Bissau [277, 278]. This high TB incidence could be due to the sub-optimal performance by DOTS and its ineffectiveness in breaking the transmission chain of the disease in the study area. Therefore, the involvement of health extension workers in raising community awareness through education may increase the possibility of capturing undiagnosed infectious TB cases, thereby improving the DOTS performance in Ethiopia in general, and in the study area in particular [238].

The estimation of TB prevalence and incidence based on symptom inquiry and sputum microscopy is a less expensive and simple technique. This method might help to generate information on the magnitude of TB in resource-constrained settings. However, it can only identify TB cases that report their symptoms. Furthermore, due to the atypical clinical presentation of HIV-TB co-infected patients, in which some may not actually report their symptoms, it is possible to obtain at low smear-positivity. Thus, using this method may undermine the actual TB prevalence and incidence in the community. Still, we used this method due to the fact that tuberculin surveys or combined chest x-rays, together with symptom inquiry and sputum microscopy, are often technically or logistically difficult in poor resource settings.

Moreover, the exclusion of 20 contaminated sputum cultures from the analysis during the baseline survey may have resulted in an underestimation of the prevalence of sputum culture TB. Likewise, 1,634 individuals who did not participate in the baseline survey were also excluded from the subsequent follow-up study due to the fact that the exact time of their contribution to the denominator was not known. However, the overall proportion of participants excluded from the follow-up study was only 5.5%, and their baseline socio-demographic characteristics were similar to those who were included in the study. Thus, their exclusion may not affect the overall findings of the study. Furthermore, some relevant variables such as HIV, which is known to be a risk factor for developing the disease, were not included in the study. We could not fully control other possible confounding variables, which may have introduce bias.

Conclusions and recommendations

Conclusions

The introduction and expansion of DOTS with improving population coverage has increased the overall TB case notification and treatment success rate (TSR), while decreasing the death and default rates over time in the study area. However, the large variations in TB case notification and treatment outcomes across the 25 districts of the zone warrant a district-specific intervention strategy. Universal achievement of the global WHO targets in infectious TB case detection and its cure rates across different geographical settings, with a diversity in socio-economic condition and health service coverage, is a challenge.

Studies in this thesis have demonstrated the importance of using existing health facilities data in providing the necessary information about the performance of TB control programmes across different districts. Therefore, the findings of these studies could help in improving the TB control programme in Ethiopia. Districts with a high TB/HIV co-infection rate have a high TB case notification. The high TB case notification observed among 15-24 years groups may indicate the ongoing transmission of TB in the community. The low TSR among re-treatment was the result of a high rate of MDR-TB among re-treated TB cases in the study area.

This study has identified a high rate of primary and secondary resistance to any of the first-line anti-TB drugs in the study area. A high rate of anti-TB drug resistance is associated with a previous history of TB treatment. Moreover, the prevalence and incidence of smear-positive and bacteriologically-confirmed TB cases were high in the study area. For every case of smear-positive TB receiving treatment, there is almost an equal number (0.96) of undetected infectious bacteriologically-confirmed TB cases in the community. The overall 15-year average PTB+ case detection rate of 37.7%, and a cure rate of 66.9% with a high proportion of undetected infectious TB cases, primary and secondary drug resistance TB in the community, could be the result from the sub-optimal DOTS performance in detecting 70% of infectious TB cases and attaining a cure rate of 85% in the study area.

Recommendations

This thesis has identified several findings that can be used to improve DOTS performance through devising an area-specific strategy in TB control programmes, while simultaneously reducing the disease burden. Based on the findings of studies in this thesis, we forward the following recommendations to TB control programmes and policy makers.

For TB control programme

- TB case findings and treatment should be further decentralized and given at health posts through using health extension workers may improve case notification and treatment compliance.
- The TB recording and reporting systems should be improved in order to avoid the under- and overestimation of the CNRs to better understand the DOTS performance across different geographic settings.
- The facility-based routine TB data reported from each district to higher levels needs to be analysed and utilized locally to help identify the gap and improve the performance of TB control programme in the districts.
- TB culture with a drug susceptibility test and GeneXpert RIF/MTB should be provided for the re-treatment of PTB patients to determine the MDR-TB status and to guide therapy.
- Strengthening DOTS and the DOTS-Plus programme with the expansion of MDR-TB diagnostic facilities could help the effort to prevent the spread of MDR-TB, and to improve the sub-optimal DOTS performance.
- Intensifying contact tracing among the household members of PTB+ cases through the involvement of community-based health extension workers and the prompt treatment of smear-positive TB cases need to be emphasized to reduce the risk of transmission in the community.

For policy makers

- Area-specific and appropriate strategies should be devised for districts with low case notifications and low treatment outcomes.
- TB control programme performance should be evaluated using trend analysis in TB case notification and treatment outcomes in order to estimate its impact on TB prevalence and incidence at the community level.
- The TB control programme performance should be periodically evaluated in relation to the trends on the prevalence of primary and secondary drug resistance, including MDR-TB, to understand its burden and to devise an applicable strategy for an effective prevention of the emergence and spread of drug-resistant TB in the country.

For research

- A high prevalence of TB cases at the baseline survey was observed among the younger age group, but shifted to the older age groups on the prospective follow-up study, which needs further investigation.
- A community-based survey on the prevalence of primary and secondary drug resistance should be carried out national wide in different urban and rural districts to determine the magnitude of the disease at the national level.
- The efficiency of using the electronic recording system in improving the recording and reporting system in the TB control programme needs to be determined.
- Determine the spatial distribution of PTB+ among the districts to find out whether there is a clustering of the disease to design a district-specific control strategy.

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Paper I

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Trends in TB case notification over fifteen years: the case notification of 25 Districts of Arsi Zone of Oromia Regional State, Central Ethiopia

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Abstract

Background: The aims of tuberculosis (TB) control programme are to detect TB cases and treat them to disrupt transmission, decrease mortality and avert the emergence of drug resistance. In 1992, DOTS strategy was started in Arsi zone and since 1997 it has been fully implemented. However, its impact has not been assessed. The aim of this study was, to analyze the trends in TB case notification and make a comparison among the 25 districts of the zone.

Methods: A total of 41,965 TB patients registered for treatment in the study area between 1997 and 2011 were included in the study. Data on demographic characteristics, treatment unit, year of treatment and disease category were collected for each patient from the TB Unit Registers.

Results: The trends in all forms of TB and smear positive pulmonary TB (PTB+) case notification increased from 14.3 to 150 per 100,000 population, with an increment of 90.4% in fifteen years. Similarly, PTB+ case notification increased from 6.9 to 63 per 100,000 population, an increment of 89% in fifteen years. The fifteen-year average TB case notification of all forms varied from 60.2 to 636 (95% CI: 97 to 127, P<0.001) and PTB+ from 10.9 to 163 per 100,000 population (95% CI: 39 to 71, p<0.001) in the 25 districts of the zone. Rural residence (AOR, 0.23; 95% CI: 0.21 to 0.26) and districts with population ratio to DOTS sites of more than 25,000 population (AOR, 0.40; 95% CI: 0.35 to 0.46) were associated with low TB case notification. TB case notifications were significantly more common among 15-24 years of age (AOR, 1.19; 95% CI:1.03 to 1.38), PTB- (AOR, 1.46; 95% CI: 1.33 to 64) and EPTB (AOR, 1.49; 95% CI; 1.33 to 1.60) TB cases.

Conclusions: The introduction and expansion of DOTS in Arsi zone has improved the overall TB case notification. However, there is inequality in TB case notification across 25 districts of the zone. Further research is, recommended on the prevalence, incidence of TB and TB treatment outcome to see the differences in TB distribution and performance of DOTS in treatment outcomes among the districts.

Keywords: TB, Trends, Case notification, Arsi zone, Ethiopia

Background

Despite the availability of effective treatment since the mid-1990s, TB remains a major public health problem and the second leading cause of death worldwide [1-3]. In 2011 there were about 8.7 million new TB cases and 1.4 million deaths worldwide from the disease [4]. In 1993, the World Health Organization (WHO) declared TB as a global public health emergency and recommended Directly Observed Treatment Short course (DOTS) as a standard strategy to control the disease [5,6]. DOTS aim to detect 70% of infectious cases and successfully treat 85% of them to interrupt the transmission, reduce mortality and prevent emergence of drug resistance [5,6].

In WHO Global TB report, Ethiopia ranked 7th among 22 High Burden Countries and 3rd in Africa in 2011 [3,7]. Moreover, TB is one of the most important infectious diseases responsible as 3rd cause of hospital admission and the second top causes of death in Ethiopia [3,8]. According to the 2011 national TB survey result, the prevalence of all forms of TB was 240 and PTB + was 108 per

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100,000 population [9]. However, studies from northern, southern and central Ethiopia [10-16] have shown that the prevalence of smear PTB + ranged from 76 to 189 per 100,000 population suggesting TB prevalence varied across different geographical locations of the country. Moreover, evidences form northern Ethiopia and other African counties have shown there is clustering of TB cases and variation in TB prevalence rate across different geographic settings [15,17-20].

DOTS was piloted in Ethiopia in the mid of 1992 in Arsi and Bale zones of Oromia regional state [21]. In 2010, it was gradually scaled up to the entire country and came up to have a 100% district and 90% health facility coverage [22]. Three studies, two from southern and one from northern Ethiopia, showed that the implementation of DOTS strategy over ten, eight and three years had improved the trends in TB case notification and treatment success with the expansion of DOTS programme to the general health services [5,23,24].

To our knowledge, no study has evaluated the progress of DOTS strategy in the trends of TB case notification and made comparison of its performance across districts in the country. In fact, Arsi was one of the nationally selected zones that piloted DOTS programme in 1992 [8]. However, performance of the programme including trends in TB case notification has not been assessed in the zone. The aim of this study was, therefore, to analyse trends in TB case notification over fifteen years and make comparisons among the 25 districts of Arsi zone of Oromia Regional State, Central Ethiopia.

Methods

Study settings

Arsi, one of the zones of Oromia Regional State, is located at 175 km southeast of Addis Ababa. It has one urban and 24 rural district with 3.1 million people residing in an area of 21,120 km^2. It is also one of the most densely populated zones with 148 people per km^2. About 89% of the population lives in rural areas while the remaining 11% resides in urban areas. In 2011 about 70% of zonal population lived within two-hour walking distance from a public health facility. According to the national standard universal health service coverage, population living within two-hour walking distance or 10 km radius of either a health centre or a hospital is considered to have access to DOTS and other health services [25]. Moreover, a hospital or a health centre with DOTS service during the study period was considered as a DOTS site. Accordingly, DOTS population coverage of each district of the zone was computed by taking population living within two-hour walking distance or 10 km radius (estimated at 250,000 for hospital and 25,000 for health centre) of a hospital or a health centre as nominator, and the total number of mid-year population of each district as denominator multiplied by 100.

Since 1997, the DOTS programme has been gradually expanding to the general health services of the zone. In 2011, DOTS strategies were fully integrated into all health facilities of the zone and used both as TB diagnostic and treatment units. These treatment units have standard TB Unit Registers from the National Tuberculosis and Leprosy Control Programme (TLCP).

Study design and data collection

This was a facility-based retrospective longitudinal study design. In this case, we reviewed the profile of all forms of TB cases registered between September 1, 1997 and August 31, 2011 to analyze the trends in TB case notification and make compression of TB performance across districts in Arsi Zone of Oromia Regional State, Central Ethiopia.

All forms of TB cases registered during the study period in all health institutions that provided DOTS services in 25 districts of the zone (73 health centres and one hospital) were included in the study. TB Unit Registers in all health facilities during the period were identified by the principal investigator and brought to the regional health bureau office between January and March 2013. Then, 10 trained data clerks collected TB patients' information on sex, age, address, TB type, patient category, date treatment started, and HIV testing and their status from the TB Unit Registers and entered the data onto a computer programme (SPSS version 20) from April to June 2013.

Definitions of terms

Based on the National Tuberculosis and Leprosy Control Programme guideline (NLCP) adopted from WHO [8], the various types of tuberculosis (TB) are defined as follows:

Pulmonary TB smear-positive (PTB+) is a patient with at least two initial sputum smear positive for acid-fast bacilli (AFB) by direct microscopy or a patient with only one sputum smear positive for AFB and with chest radiographic abnormalities consistent with active pulmonary TB followed by clinician's decision.

Pulmonary TB smear-negative (PTB-) is a patient with at least three initial sputum smear negative for AFB by direct microscopy and with chest radiographic abnormalities consistent with active pulmonary TB and no clinical response to two weeks of broad spectrum antibiotic therapy followed by clinician's decision.

Extra pulmonary tuberculosis (EPTB) is tuberculosis involving organs other than the lungs, such as skin, abdomen, joints and bones, lymph nodes, pleura, genitourinary tract, and meninges. The diagnosis is based on fine needle aspiration (FNA) for histopathological examination or biochemical analysis of ascetic/pleural/cerebrospinal fluid followed by clinician's decision to treat it with a full course of anti-TB drugs. However, a patient with three initial sputum smear negative for AFB at health centre and with no clinical response to two weeks of broad spectrum

antibiotic therapy, and also suspected of EPTB at health centre were referred to hospital for further radiological and histopathological investigation before diagnosis as TB cases at health centre level.

Measurements

Area of residence, sex, age, HIV status, type of TB, patient category and population ratio to health facilities with DOTS service [25-28] were used as independent variables, whereas TB case notification was taken as dependent variable. The independent and dependent variables were further categorized into groups for analysis.

The population size used as denominator to calculate TB case notification of 25 districts of the study area was obtained from the 1997 and 2007 National censuses [29,30]. The mid-year population of each district for each of the fifteen years was then extrapolated from the two censuses. The fifteen-year average mid-year population of each district was obtained by adding the mid-year population for each of the fifteen years (1997 to 2011) in each district and then dividing it by 15. The fifteen-year average of all forms of TB case for each district was also computed by adding all forms of TB case notified by each district in each year (between 1997 and 2011) and dividing it by 15. The same procedure was followed to compute the fifteen-year average of PTB + case for each district. Finally, the 15-year average of all forms of TB case notification and PTB + case notification for each district was computed by dividing the 15-year-average of all forms of TB and PTB + cases notified in each district by the 15-year-average mid-year population of their respective district and multiplying it by 100,000 population.

$$\text{Fifteen-year ATBCN} = \left(\frac{\text{15-year average of all TB case notified in each district}}{\text{15-year average mid year population of their respective districts}}\right) \times 100,000 \text{ population}$$

NB: ATBCN is fifteen-year average of TB case notification

The PTB + case detection rate (CDR) for each year (1997 to 2011) of the zone was computed as follows: first we calculated the total number of expected incidence of PTB + cases for each mid-year population of the zone (104/100,000 for Oromia) based on the 2011 National TB Prevalence survey result where Arsi zone was one of the zones in the region [9]. Then we calculated the PTB + CDR by taking the total number of PTB + cases notified in each year in the zone as nominotor and the total expected PTB + incidence cases of the zone for each year as denomintor and multiplying it by 100.

$$\text{PTB + CDR for each year (1997 to 2011)} = \frac{\text{Total number of PTB + case notified in each year in the zone}}{\text{Total number of expected PTB + case in each year in the zone}} \times 100$$

Since 2008, all TB patients have been offered provide initiated voluntary counselling and testing service for HIV. Hence, TB-HIV co-infection rate for each district was computed by taking all TB patients tested for HIV and found to be HIV positive as nominator and all TB patients tested for HIV during the study period as denominator multiplied by 100.

Statistical analysis

Data were coded and double entered by trained data clerks using Epi-info statistical software version 7. Later, they were exported to IBM SPSS version 20 for data checking, cleaning, and bivariate and multiple logistic regression analysis. Descriptive analyses such as frequency, mean, and standard deviation were computed as appropriate. Adjusted odds ratio was used to determine the strength of association between the study variables at 95% CI and P value <0.05. The model adequacy and co-linearity assumptions were checked to be satisfied based on appropriate methods designed for the study.

In the bivariate and multivariate binary logistic regression analyses of TB case notification, we excluded Assela town (urban district) from the analysis of fifteen-year average of all forms of TB case notification of districts in the zone. This was because Assela Referral Hospital in Assela town was used as referral center for TB cases from the entire zonal population and the neighboring zones. Therefore, if we take the population of the town as a denominator to calculate its TB case notification, we might overestimate the TB case notification of the zone and the town. Consequently, after excluding Assela town form the analysis, the fifteen year-average of all forms of TB case notification of the 24 rural districts of the zone was found to be 120/100,000 population and the study subjects were normally distributed. Therefore, we took this as cut-off value in the dichotomization of all forms of TB case notification into below 120 and above 120 per 100,000 in the bivariate and multivariate binary logistic regression analyses.

Ethical approval

Ethical approval was obtained from institutional Review Board of Oromia Regional State Health Bureau, Ethiopia. Formal permission to use the data was obtained from the officials.

Results

General characteristics of the study subjects

A total of 41,965 TB patients were registered in 74 treatment units of Arsi zone between September 1, 1997 and

August 31, 2011. Gender wise, more than half, 22,743 (54.2%), were males and residence wise, slightly over a third, 14,052 (36%) were urban residents. The age of the patients ranges from one to 98 years with mean age of 28.7 years (SD +15.3 years). The majority (93%) were new and over a third of them, 15,370 (36%) were pulmonary smear-positive; 15,102 (36%) were pulmonary smear negative; and 11,447 (27.3%) were extra-pulmonary tuberculosis cases (Table 1).

The Trends in DOTS Site Expansion and TB Case Notification

The trend in TB case notification was significantly associated with the number of DOTS sites in the zone (Figure 1a and 1b, $X^2_{trend} = 75.2$, $P < 0.001$) which gradually increased from five in 1997 to 23 in 2001 and from 46 in 2007 to 74 in 2011 (Figure 1b). Similarly, the DOTS population coverage increased with the number of DOTS sites. It went from 18% in 1997 to 36% in 2001 and from 52% in 2007 to 70% in 2011 (Figure 1c). Subsequently, with increased number of DOTS sites, all forms of TB case notification of the zone increased from 14.3 in 1997 to 150 per 100,000 population in 2011, an increment of 91% in fifteen years. Figure 1a also shows there was a steady upward trend in all forms of TB case notification in the first four years of DOTS introduction (from 14.3 in 1997 to 96.5 per 100,000 in 2000). However, this rising trend has been stabilized in the range between 91.2 and 104 per 100,000 population between the years 2001 and 2006. The trends further increased again to 128 in 2007 and to 150 per 100,000 population in 2011.

Table 1 General characteristics of the study subjects (n = 41,965), Arsi Zone, Oromia Regional State, Central Ethiopia, 1997-2011

Patient Characteristics	Number	Percentage
Age Category		
0-14 years	5,587	13.3
>14 years	36,004	85.8
Unknown	374	0.9
Sex		
Male	22,743	54.3
Female	18,815	44.8
Unknown	382	0.9
Patient Category		
New	39,010	93.0
Relapse	804	1.9
Failure	61	0.1
Defaulter	99	0.2
Transfer-In	894	2.1
Other Cases	705	1.7
Unknown	392	0.9
TB Classification		
Pulmonary/Positive	15,370	36.6
Pulmonary/Negative	15,102	36.0
Extra-pulmonary	11,447	27.3
Missing (unknown)	46	0.1

Likewise, PTB + case notification of the zone increased from 7 in 1997 to 63 per 100 000 population in 2011 with an increase of 89% in fifteen years. Similarly, the trend in TB case detection rate (CDR) of the zone went up from 6.4% in 1997 to 34.5% in 2001, and from 48.3% in 2007 to 58.7% in 2011 ($X^2_{trend} = 26.8$, $P < 0.001$) (Figure 1c). Similar to the trend in all forms of TB case notification, PTB + in the first four years of DOTS introduction increased from 6.9 in 1997 to 41 per 100,000 population in 2000. However, through the years 2001 to 2010, with the exception of 2007, the trend stabilized in the range between 35 and 46 per 100,000 population (Figure 1a).

Fifteen-year average TB case notification by districts

Table 2 shows that fifteen-year average TB case notification across the 25 districts of the zone was unevenly distributed. All forms of TB notification were high (over 145 per 100,000 population) for seven districts and low (below 85 per 100,000) for six districts (Figure 2). The highest all forms of TB case notification was from Assela town (636.1 per 100,000) followed by Dodota district (314.2 per 100,000) (Table 2). Similarly, TB and HIV co-infection in Assela town and Dodota district was 24.2% and 16.4% respectively, high compared to other districts. Nevertheless, the lowest (63 per 100,000) all forms of TB case notification was observed in Tiyo and Aseko where TB and HIV co-infection was 6.9% and 2% respectively. This is less than 9.4% of the zonal average TB and HIV co-infection (Table 2).

Similarly, the 15-year-average of PTB + case notification for the 25 districts of the zone was 42.3 per 100,000 population. Table 2 and Figure 2 show that the fifteen-year average PTB + case notification for five districts was high and above 50 per 100,000 population compared to other seven districts with case notification of less than 20 per 100,000 population. As observed in all forms of TB, the highest fifteen-year average PTB + case notification was observed in Assela town (163 per 100,000) followed by Dodota district (150.2 per 100,000 population). The lowest was from Tiyo (10.9 per 100,000) preceded by Munessa (14.9 per 100,000 population) (Table 2).

In binary logistic regression analyses, area of residence, age, type of TB and population ratio to DOTS sites of the districts were associated with low TB case notification. In the final model, rural residence (AOR, 0.23; 95% CI: 0.21 to 0.26) and districts with population ratio to DOTS sites with more than 25,000 population (AOR, 0.40; 95% CI: 0.35 to 0.46) were associated with low TB

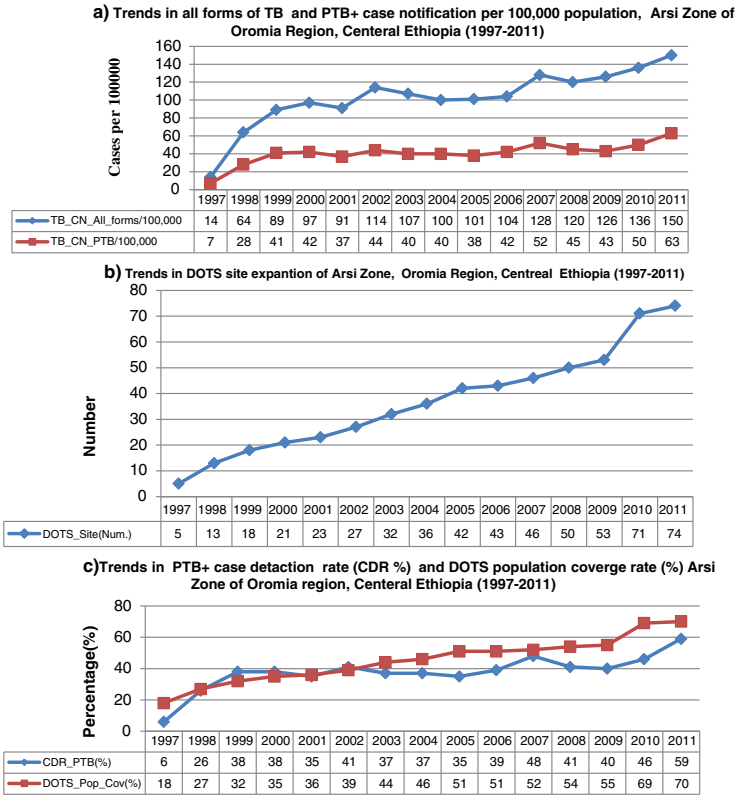

Figure 1 Trends in all forms of TB, Pulmonary smear positive TB case notification, DOTS sites expansion and DOTS population coverage, Arsi Zone of Oromia Regional State, Central Ethiopia, 1997-2011. N.B: Trends in TB case notification increased with DOTS sites expansion for all forms of TB X^2_{trend} =75.2, p<0.001, PTB+ X^2_{trends} =27.74, P <0.001) and CDR PTB+ X^2_{trend} =26.794, p<0.001.

case notification. However, TB case notifications were significantly more common among 15-24 years of age (AOR, 1.19; 95% CI: 1.03 to 1.38), PTB- (AOR, 1.46; 95% CI: 1.33 to 64) and EPTB (AOR, 1.49; 95% CI; 1.33 to 1.60) TB cases (Table 3).

Discussion

The study has confirmed that the expansion of DOTS strategy led to improved TB case notification across 25 districts of Arsi zone. However, it was identified that there is inequality and uneven distribution in TB case notification among the districts. The new knowledge about difference in TB case notification across districts is an important indication calling for investigation to identify if this difference is related to difference in the prevalence of the disease or to the existence of undetected TB patients among districts with low TB case notification.

These findings are significant particularly in resource-constrained settings where there is limited health infrastructure and inadequate physical and financial access across different geographical settings. If this inequality in TB case notification resulted from access to health care, then it may limit the effectiveness of DOTS strategy to attain the global WHO 70% TB case detection rate and 85% treatment success rate aimed at interrupting TB transmission, reducing mortality and preventing emergence of drug resistance [5,31].

The DOTS strategy was initially introduced in 1992 as a national pilot in a health centre and a hospital in Arsi zone [8,21] followed by systematic scaling up of the control programme to other health facilities. This stepwise DOTS expansion started in health facilities at the district capital and then continued to sub-district level health facilities. Meanwhile, with the exception of health facilities

Table 2 Yearly average TB case notification/100,000 population and TB/HIV co-infection rate (%), 25 districts in Arsi Zone of Oromia region, Centeral Ethiopia (1997-2011)

Name of district	Average fifteen-year mid-year population	No. of TB cases notified (Avg.)		TB case notification/100,000		TB-HIV co-infection	
		All forms	PTB+ve	All forms	PTB+	Number of tested	HIV positive (%)
Tiyo	80,384	50	9	62.5	10.9	130	9 (6.9%)
Amigna	67,862	61	11	90.3	16.3	178	18 (9.6%)
Aseko	77,930	49	23	63.2	29.6	304	6 (2%)
Assela town	62,325	396	102	636.1	163	1415	342 (24.2%)
Bele/Ge	68,517	84	18	122.9	25.7	167	6 (3.6%)
Cholle	82,728	71	27	85.4	33	282	30 (10.6%)
Digalu/Tijo	130,142	207	70	159	53.7	838	62 (7.4%)
Diksis	66,987	93	26	138.8	38.1	224	5 (2.2%)
Dodota	59,583	187	90	314.2	150.4	444	73 (16.4%)
Gololcha	159,521	139	28	86.8	17.5	491	7 (1.4%)
Guna	70,752	58	30	81.3	42.2	240	12 (5%)
Hetosa	115,089	187	56	162.9	48.4	688	63 (9.2%)
Honkolo/Wa	54,257	79	11	145.1	20.2	122	3 (2.5%)
Jaju	114,972	155	59	135.2	51.1	788	34 (4.3%)
Limu/Bibi	167,414	146	63	87	37.8	429	46 (10.7%)
Lode Hitosa	99,259	146	43	146.7	43	433	61 (14.1%)
Merti	83,763	143	42	171.1	50.1	595	56 (9.4%)
Munessa	154,299	165	23	106.7	14.9	423	42 (9.9%)
Robe	153,067	205	68	133.8	44.4	517	30 (5.8%)
Shirka	151,782	116	37	76.5	24.6	211	7 (3.3%)
Sire	68,533	98	33	142.4	48.1	395	27 (6.8%)
Sude	136,904	112	27	82	19.9	293	3 (1%)
Tena	61,337	37	10	68.8	16.4	206	14 (6.8%)
Z/Dugda	111,979	128	20	87.2	17.9	412	16 (3.9%)
Seru	44,406	52.2	11	82.5	40.5	203	12 (5.9%)

under construction, full DOTS service coverage was achieved in eight years. This goes in line with previous reports from Southern Ethiopia where 73% of zonal [23] and full zonal and districts [5] DOTS service coverage were achieved in seven years following the introduction of DOTS strategy.

The continuous DOTS-site expansion in Arsi zone over the past fifteen years might be due to high political commitment in securing necessary resource for the establishment of laboratory network for effective TB diagnosis, treatment, and monitoring, and in ensuring an uninterrupted supply of anti-TB drugs [5,32].

Consequently, between 1997 and 2011, TB case notification increased from 14 to 150 for all forms and from 6.9 to 63 per 100,000 population for PTB+. This finding substantiates results of previous studies in Southern Ethiopia where the ten-year trend in all forms of TB case notification increased from 45 to 143 [5] and PTB + from 49 to 126 per 100,000 population in eight years [23]. However, our study showed that under the current passive TB case findings, the trend in TB case notification did not persistently increase though out the study period. This might warrant the involvement of health extension workers in active TB case finding in the Ethiopian context so as to achieve the MDG of 70% of TB case detection rate [33].

The case detection rate (CDR), estimated by the proportion of PTB + cases notified form the total expected PTB + incidence cases in the community, showed an increase from 7% in 1997 to 63% in 2011. Previous studies [5,23] also indicated that the trends in PTB + CDR more than doubled in eight years. Likewise, in Vietnam the CDR increased by more than six-fold in fifteen years [32]. The most likely explanation for the increase in CDR over time could be due to the real increase in PTB + case detection rate following decentralization of DOTS strategy led to improved access to laboratory service.

The other reason could be the improvement in recoding and reporting of detected TB cases following the

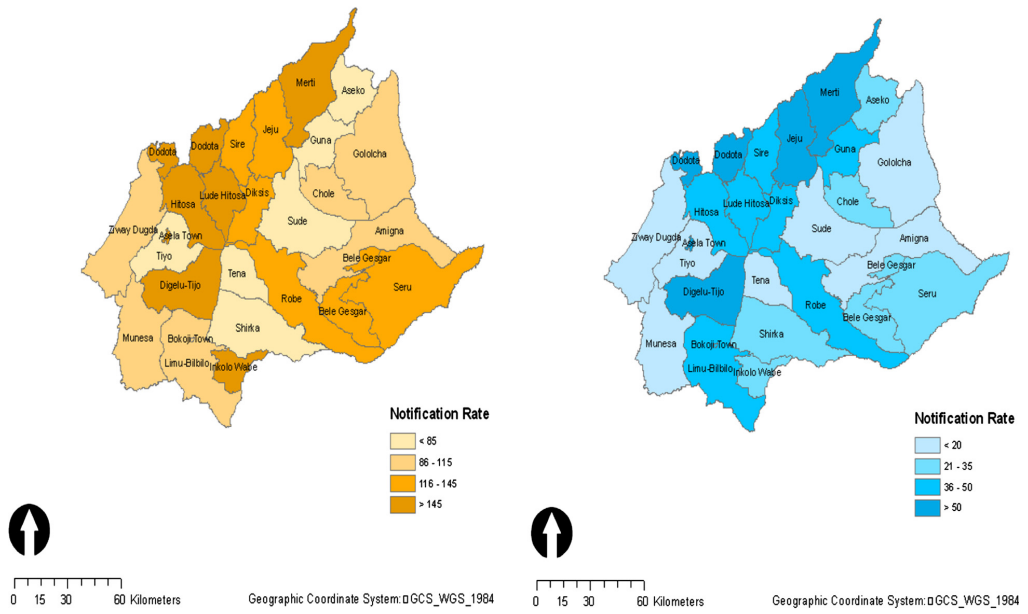

Figure 2 Fifteen-year average of all forms and PTB+ve TB case notification of 25 districts of Arsi Zone, 1997-2011.

introduction of DOTS without real increase of TB case detection rate [34]. Nevertheless, the increased trend might also be due to true increase in TB incidence cases fueled by the powerful interaction between HIV and tuberculosis [35]. The TB and HIV co-infection among tested TB patients in our study was 9.4%. It might also be due to the notification of large backlog of TB cases that resulted from improved TB diagnostic access [34].

The upward trend in CDR in this study was slightly higher than report from Southern Ethiopia [5] but lower than other reports from the same region and Vietnam [23,32]. The explanation for this discrepancy might be due to variation in DOTS performance across different study areas. The difference could also be due to the variation in disease burden across different geographical settings [15,17-20].

The PTB + case notification and CDR of the zone steadily increased during the first six years of DOTS implementation. However, despite a notable increase in the number of TB diagnostic centres, the PTB + case notification and CDR seem to be stable during the years 2002–2010. The result corroborates the previous report that indicated the number of reported TB cases did not proportionally increase with the number of TB DOTS sites after five years of DOTS introduction [5]. Moreover, the finding confirms pervious study reports where an increase in the trends of PTB + case detection rate in the first five years [5,23] and seven years [32] of DOTS introduction was followed by stabilizing of case detection rates. The expansion of DOTS-sites may help to improve case notification to a certain point while increase in coverage may contribute to marginal increment in case finding unless other community level interventions are introduced [33].

In the mathematical model used to predict the WHO target of 70% TB case detection rate and 85% cure rate in countries where the incidence of tuberculosis is stable and HIV-1 absent, there would be a reduction of TB incidence rate by 11% and death rate due to TB by

Table 3 Factors associated with case notification rate among patients registered from 1997-2011 in Arsi Zone, Oromia Regional State, Central Ethiopia

Variables	Category	Category of case notification	COR (95% CI)	AOR (95% CI)	
		<120 cases/100,000	>120 cases/100,000		
Residence (N = 39,471)	Urban	2,554 (17.5)	11,598 (82.5)	1.00	1.00
	Rural	9,310 (36.8)	16,009 (63.2)	0.36 (0.35,0.38)**	0.23 (0.21,0.26)**
Sex (N = 41,583)	Male	6,876 (30.2)	15,892 (69.8)	1.00	1.00
	Female	5,716 (30.4)	13,099 (69.6)	0.99 (0.95,1.03)	0.99 (0.89,1.06)
Age (N = 41,591)	0-14 years	1,676 (30.0)	3,911 (70.0)	1.00	1.00
	15-24 years	4,059 (31.2)	8,942 (68.8)	0.94 (0.88,1.01)	1.19 (1.03,1.38)*
	25-49 years	5,387(29.7)	12,729 (70.3)	1.01(0.95,1.08)	1.10 (0.96,1.28)
	≥50 years	1,515 (31.0)	3,372 (69.0)	0.95 (0.88,1.04)	1.09 (0.92,1.39)
TB Type (41,919)	PTB+	5,226 (34.0)	10,144(66.0)	1.00	1.00
	PTB -	3,904 (25.9)	11,198 (74.1)	1.48 (1.41,1.55)**	1.46 (1.33,1.64)**
Population ratio to DOTS sites in the districts (N = 41,965)	EPTB	3,634 (31.7)	7,813 (68.3)	1.11 (1.05,1.17)**	1.49 (1.33,1.6)**
	≤ 25,000	1,112 (16.3)	5,713 (83.7)	1.00	1.00
	25,001-40,000	8,280 (43.6)	10,713 (56.4)	0.25 (0.24,0.27)**	0.41 (0.36,0.47)**
		3,400 (21.1)	12,747 (78.9)	0.73 (0.68,0.79)**	0.40 (0.35,0.46)**

NB: - * Significant at P-value<0.05 and ** Significant at P-value<0.001.
With the national standard that one health centre with DOTS service can be accessible for 25,000 in TB care, the ratio of population size to the number of DOTS sites was calculated as total number of population in the district divided by number of DOTS sites in the same district. (Total number of population in the District/ Total number DOTS sites in the same district).

12% per year [36]. However, in this study where there is low PTB + CDR (63%) and high prevalence rate of HIV among TB patients (9.4%), it is not convincing to argue that the decline in TB incidence led to stability in PTB + case detection rate after six years of DOTS implementation.

In this study, we identified variations in TB case notifications among 24 rural districts and one urban district of Arsi zone. The findings show that TB case notification of Dodota district among the 24 rural districts was very high compared to Tiyo, Amigna, Shirka, Sude and Tena districts. Such a situation in Dodota district might be explained by the true increase in TB incidence cases fueled by the powerful interaction between HIV and tuberculosis [35] as TB and HIV co-infection of the district is very high (16.4%) compared to 9.4% of zonal average.

The overall fifteen-year average PTB + case notification of the districts was 42.3 per 100,000 population with a fourteen fold variation between different districts (10.9 to 150 per 100,000 in rural and 166 per 100,000 in urban areas). This dissimilarity in PTB + case notification among districts may be an indication of inequity in TB case findings or heterogeneity in TB incidences across 25 districts [15,17,18,37] caused by diversity in TB risk factors [32,38]. This difference could be a result of the defect in the current passive facility-based TB case findings of DOTS policy in uniformly notifying TB cases across districts either due to limited ability of the health system to detect TB cases or poor health care seeking behaviour particularly for those with TB [20].

In this study factors like area of residence, age of patients, type of TB and the ratio of population size to DOTS sites were found to be associated with the level of TB case notification. This is in agreement with studies conducted elsewhere [26-28] where TB case notification was associated with urban residence, age of patient, access in TB care and type of TB.

Although our study demonstrated the usefulness of facility-based data analysis at district level, it has some limitations. This retrospective facility-based longitudinal study has no socio-economic and environmental data which is the inherent limitation of retrospective study. Therefore, the absence of socio-economic and environmental data in this study could affect the result as it might have an association with the variation in TB case notification. There might also be bias in TB case notification of the districts due to the extrapolation of census data from 1997 and 2007 to get up-to-date denominator of the population. However, there might be unevenly distributed population growth rate across years of the study period and districts. If this were the case, there would be either under or overestimation of TB case notification. The notification in some districts might be overestimated due to existence of some health facilities with good history of TB care that could attract more patients from nearby districts.

Conclusions

The introduction and expansion of DOTS in Arsi zone has improved the overall TB case notification. However, there is inequality across the 25 districts of the zone. Universal achievement of the global WHO target of 70% case detection rate across different geographical settings with diversity in socio-economic condition will continue to be a challenge. This study demonstrated to public health professionals the importance of using existing health facilities data in providing necessary information about TB case notification across different districts. Thus, it is important to enable the concerned bodies to try out more locally applicable and effective strategies to attain MDG in TB control. To this end, further research is recommended on the prevalence and incidence of tuberculosis and also on TB treatment outcome to see the differences in the distribution of the disease and performance of DOTS strategy in treatment outcomes across the 25 districts.

Competing interests

The author's declare that they have no competing interests.

Authors' contributions

SDH was the principal investigator who participated in designing and conducting the study. Further, he was involved in analyzing the data and writing the manuscript. BL took part in the design of the study, analysis of data and write up of the manuscript and MD participated in designing the study and writing the manuscript. All authors read and approved the final manuscript.

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Paper II

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II

Global Health Action

COACTION PUBLISHING

ORIGINAL ARTICLE

Fifteen-year trend in treatment outcomes among patients with pulmonary smear-positive tuberculosis and its determinants in Arsi Zone, Central Ethiopia

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Background: Directly Observed Treatment Short course (DOTS) strategy is aimed at diagnosing 70% of infectious tuberculosis (TB) and curing 85% of it. Arsi Zone of Ethiopia piloted DOTS strategy in 1992. Since then, the trend in treatment outcomes in general and at district-level in particular has not been assessed. The aim of this study was to analyse the trend in TB treatment outcomes and audit district-level treatment outcomes in the 25 districts of Arsi Zone.

Design: A retrospective cohort study design was employed to audit pulmonary smear-positive (PTB+) patients registered between 1997 and 2011. Demographic and related data were collected from the TB unit registers between January and March 2013. The 15-year trend in treatment outcomes among PTB+ patients and district-level treatment outcomes was computed.

Results: From 14,221 evaluated PTB+ cases, 11,888 (83.6%) were successfully treated. The treatment success rate (TSR) varied from 69.3 to 92.5%, defaulter rate from 2.5 to 21.6%, death rate from 1.6 to 11.1%, and failure rate from 0 to 3.6% across the 25 districts of the zone. The trend in TSR increased from 61 to 91% with the increase of population DOTS coverage from 18 to 70%. There was a declining trend in defaulter rate from 29.9 to 2.1% and death rate from 8.8 to 5.4% over 15 years. Patients aged 25–49 years (Adjusted Odd Ratio (AOR), 0.23; 95% CI: 0.21–0.26) and ≥50 years (AOR, 0.43; 95% CI: 0.32–0.59), re-treatment cases (AOR, 0.61; 0.41, 0.67), and TB/HIV co-infection cases (AOR, 0.45; 95% CI: 0.31–0.53) were associated with unsuccessful treatment outcomes.

Conclusions: DOTS expansion and improving population DOTS coverage in Arsi has led to a significant increase in treatment success and decrease in death and defaulter rates. However, there is a major variation in treatment outcomes across the 25 districts of the zone, so district-specific intervention strategy needs to be considered. The low TSR among re-treatment cases might be due to the high rate of MDR-TB among this group, and the issue needs to be further investigated to identify the extent of the problem.

Keywords: *tuberculosis*; *trends*; *treatment outcomes*; *Arsi Zone*; *Ethiopia*

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Although effective treatment has been used to treat tuberculosis (TB) for several decades, TB remains a major global health problem and the second leading cause of death worldwide. In 2010, from the estimated 8.8 million incidents of TB cases, 5.7 million TB cases and 1.4 million deaths were reported globally (1, 2). It was also found out that poor adherence and irregular TB treatment leads to an increase in the period of infection with the consequences of multi-drug resistance TB (MDR-TB) (3, 4).

The World Health Organization (WHO) recommended Directly Observed Treatment Short course (DOTS) (5, 6), which aims at detecting 70% of infectious cases and curing 85% of them. This strategy is expected to interrupt transmission of the disease and reduce the number of infected individuals and the period of infectiousness (4).

In 2011, Ethiopia ranked seventh among the 22 high burden countries in terms of estimated number of TB cases (7, 8). Moreover, TB is the third leading cause of hospital admission and the second cause of deaths in Ethiopia

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(7, 9). In 1992, DOTS strategy started in Ethiopia where Arsi and Bale zones of Oromia region were the first two to be selected for piloting. Since 1997, DOTS has been scaled up to include the entire country (10).

Different studies from southern and northern parts of Ethiopia show that the implementation and expansion of DOTS strategy improved the TB treatment success rate (TSR) and reduced defaulter rates (5, 11–13). Nevertheless, a study from northern Ethiopia indicated that TSR under DOTS strategy was low with high proportion of deaths and defaulters (14).

Although Arsi was the first zone to start DOTS strategy in Ethiopia, no investigation has been made to see if there are differences in TB treatment outcomes across the districts in the zone and also the trend in TB treatment outcomes over the years. Few studies analysed the trends in treatment outcomes as aggregate at either regional or national level. However, to the knowledge of the authors, none of these studies investigated district-specific treatment outcomes to see if there is variation in treatment outcomes across districts in the country (5, 11, 12, 14, 15). Hence, the aim of this study was to analyse trends in TB treatment outcomes over 5 years in the 25 districts of Arsi Zone and to investigate if there were differences in the outcome across the districts.

Methods

Study setting

The study was conducted in the Arsi Zone, Central Ethiopia. The zone has one hospital and 73 health centres in 25 districts with a total population of 3.1 million. About 89% of the zonal population resided in rural areas. In 2011, about 70% of the population lived within a 10-km radius or at a walking distance of 2 hours from a health institution and thus had access to DOTS service (16). In 2004, the government of Ethiopia, under the health extension package programme (HEP), launched a community-based essential health service to the community. The HEP is implemented through the deployment of health extension workers (HEWs) at a community level (17). A 1-year undergraduate and two new female cadres are deployed as community health workers at every *kebele* (the smallest government administrative unit) with the responsibility of providing essential health services for a population of 5,000. The objective of HEP is to ensure equitable access to health services, prevent major communicable diseases, and promote health in the community. As part of their role in the prevention of communicable diseases, HEWs are trained on how to identify and refer TB suspects, provide health education and treatment, and trace defaulters (18).

Since 1997, DOTS programme, which started piloting in one health centre and a hospital, has significantly expanded and subsequently gained full integration into 74 health facilities at the end of 2011. All health facilities were utilised as TB diagnostic and treatment units used standard TB unit registers from the National Tuberculosis and Leprosy Control Programme (NTLCP) to register TB cases. Since 2008, TB patients were offered services of provider initiated voluntary counselling and testing for HIV.

The TB drugs used in combination were isoniazid (H), rifampicin (R), pyrazinamide (Z), ethambutol (E), streptomycin (S), and thioacetazone (T). However, since 1997 thioacetazone was removed from the system and not used any more. Using the NTLCP guidelines (9), all new patients were treated with RHZ + E or S for 2 months followed by EH or RH for 6 months. The first 2 months of intensive phase treatment were under direct supervision of the health workers. During this phase, with the exception of those who were critically sick, TB patients received treatment on an ambulatory basis. Re-treatment cases were treated with SERHZ for 2 months, ERHZ for another 1 month, and ERH for 5 months (9).

Study design and data collection

Retrospective cohort study design was employed to audit TB treatment outcomes for PTB+ patients treated in the 25 districts of Arsi Zone. Pulmonary smear-positive TB cases registered between September 1, 1997, and August 31, 2011, for TB treatment in all public health institutions (73 health centres and one hospital) were included in the study. The principal investigator identified all TB unit registers used in each TB treatment unit during the study period. The total number of TB unit registers in each TB treatment unit over the study period was checked against the total number of annually reported TB cases from each TB treatment unit to the district health office to see if there was any missing TB unit register. Indeed, no missing TB unit registers were detected during the study period. After the identification of all TB unit registers from TB treatment units, the principal investigator collected them between January and March 2013. Ten trained data collectors gathered from TB unit registers socio-demographic and related data like type of TB, TB patient category, contact person for tracing, date of treatment initiation, drug regimen, treatment follow-up, follow-up sputum smear microscopic result, HIV status, treatment outcomes, and date DOTS started in each health institution.

Definitions of terms

Type of TB and treatment outcome were defined according to the NTLCP guideline adopted from WHO and a previous study report (15, 18).

Measurements

The area of residence, sex, age, HIV status, TB patient category, treatment regimen, and history of contact person for tracing were taken as independent variables

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and treatment outcomes as dependent variable. The dependent variable (15-year average treatment outcomes) of each district was computed from the sum of annual TSR of 15 years for each district (nominator) divided by 15 (denominator), which is the number of years of the study period. The trend in PTB+ treatment outcomes of the zone was computed from the sum of the annual PTB+ treatment outcomes of the 25 districts in the zone for each year of the study period. Comparison of 15-year average treatment outcomes among districts was also made after computing the 15-year average of treatment outcomes for PTB+ cases for each district in the zone. Moreover, a comparison of treatment outcomes between the new and the re-treated PTB+ cases was also made.

Population DOTS coverage of the zone was also calculated as the total number of the population residing within a 10-km radius or 2 hours walking distance from a public health institution as nominator and the total number of population of the zone in each year as denominator multiplied by 100. The National Health Service standard where one health centre is expected to serve 25,000 people and one hospital to serve 250,000 people was used to calculate population DOTS coverage (19).

A total of 521 PTB+ patients with incomplete records on their treatment information and 628 who were transferred out to other health institutions out of the zone were excluded from the analysis (Fig. 1).

Statistical analysis

Data were coded and double entered by 10 trained data clerks using Epi-Info version 7. We used IBM SPSS version 20 for data checking, cleaning, and analysis. Descriptive analyses such as frequency, mean, and standard deviation were computed as appropriate. The analyses of linear trend for TSR, cure rate, defaulter rate, and death rate were analysed, and statistical significance was cheeked using X^2 for trend. Bivariate and multivariate logistic regression analysis was used to determine the association between independent and dependent variables. Variables with $p < 0.2$ in bivariate analysis were fitted into the final multiple logistic regression models. Variables with $p < 0.05$ in the final model were taken as significant determinants. The model adequacy and co-linearity assumptions were checked using F-test and assessed for normality by displaying continuous data on a histogram. All numerical data were found to be normally distributed. Multi-co-linearity of the independent variables was

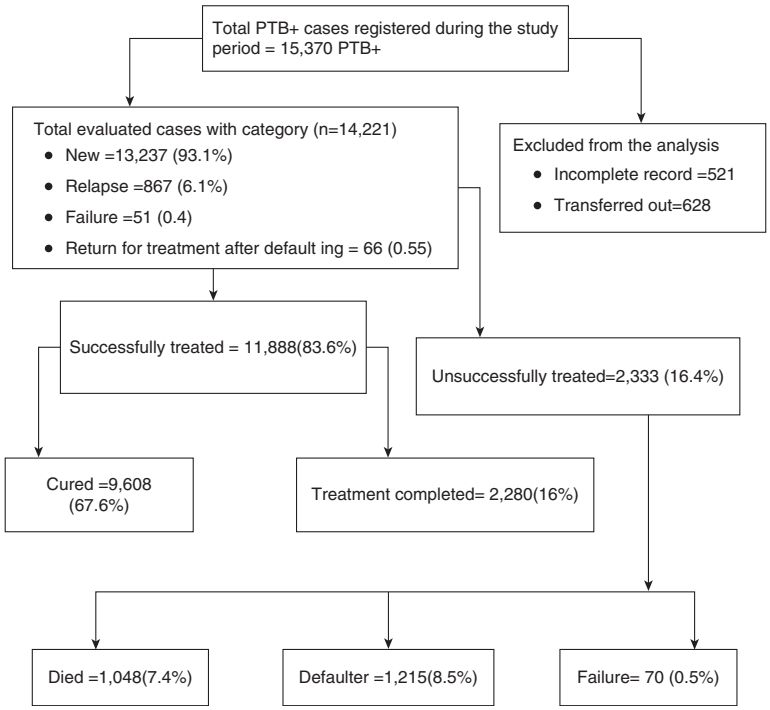

Fig. 1. Profile for pulmonary smear-positive TB patients registered during 1997–2011 and treatment outcomes, Arsi Zone, Central Ethiopia.

assessed using Pearson correlation, and those with r values of 0.6 or less were used in model fitting.

Ethical approval

An Institutional Review Board of Oromia Regional State Health Bureau, Ethiopia, approved the research for scientific and ethical integrity. Formal permission to use data in the study was obtained from the heads of Zonal and District Health Offices and Health Institutions.

Results

Patient characteristics

A total of 15,370 PTB+ patients were registered in 25 districts of Arsi Zone between September 1997 and August 2011. Of these, the treatment outcomes of 14,221 (92.5%) were evaluated. The treatment outcomes of 521 PTB+ patients with incomplete records and 628 transferred out, and hence with unknown treatment outcomes, were excluded from the analysis. From the total evaluated TB cases, 7,734 (54.4%) were males while the remaining were females, and 5,119 (34%) were urban residents. The age of the patients ranged from 1 to 98 years with a mean (standard deviation) of 28.7 (15.3%) years. The majority, 13,237 (93.1%), of the TB patients were new whereas 867 (6.1%) were relapse; 51 (0.4%) were failure; and 66 (0.5%) were return after default. This makes a total of 984 (6.9%) re-treatment cases (Table 1 and Fig. 1).

Trend over time

The trends in the TB TSR among PTB+ cases increased from 61.3% in 1997 to 91.2% in 2011 with an increase in population DOTS coverage from 18% in 1997 to 70% in 2011. The overall TSR increased by 30% and population DOTS coverage by 52% over 15 years (X^2 trend = 31. 08, $p < 0.001$). The TSR and cure rate steadily increased with DOTS site expansion through the years between 1997 and 2001 with the exception of 1999 when a decline was observed. However, the increasing trend over time in TSR stabilised in the range between 82.1 and 84.2% during the years 2003–2008 and then increased to 91.7% in 2011 (Fig. 2).

The trend in death rate among PTB+ cases remained in the high range of 12.5–8.8% during the first 7 years (1997–2005) of DOTS implantation in the study area. The highest death rate (12.5%) was observed in 1999 followed by 11.8% in 2000. However, the trend in death rate gradually declined from 5.7 to 3.9% during the last 5 years of the study period (2008–2011). The trend in defaulter rate also declined steadily from 29.9 to 2.1% over 15 years (1997–2011) (X^2 trend = 18.56, $p < 0.001$) (Fig. 2).

Treatment outcomes

The treatment outcomes of 14,221 pulmonary smear-positive TB cases were evaluated. Of these, 11,888 (83.6%) were treated successfully, 9,608 (67.5%) were cured, and 2,333 (16.4%) were treated unsuccessfully. From those treated unsuccessfully, 1,048 (7.4%) died, 1,215 (8.5%) defaulted, and 70 (0.5%) had treatment failure (Table 2).

Table 1. General characteristics of pulmonary smear-positive TB cases registered for treatment between 1997 and 2011, Arsi Zone, Central Ethiopia

Characteristics	Number	Percentage
Sex		
Male	7,734	54.4
Female	6,487	45.6
Age group		
0–14	1,939	13.6
15–24	4,389	30.9
25–49	6,207	43.6
≥ 50	1,686	11.9
Mean age (Standard Deviation)	28.7 (15.3)	
Area of residence		
Urban	5,119	34
Rural	9,102	66
Patient category		
New	13,237	93.1
Relapse	867	6.1
Failure	51	0.4
Return for treatment after defaulting	66	0.5
TB/HIV co-infection (n = 3,627 tested) by type of TB	288	7.9
Treatment outcomes		
Cured	9,608	67.5
Treatment completed	2,280	16
Successfully treated (cured and treatment completed)	11,888	83.6
Died	1,048	7.5
Defaulted	1,215	8.5
Failed	70	0.5

The TSR was higher (84.8%) among new PTB+ patients compared to re-treatment cases (67.5%). This was mainly due to the high rate of death (11.7%), defaulter (19.2%) and failure (5.6%) among the re-treatment cases. Pulmonary smear-positive new TB cases had higher TSR compared to re-treated PTB+ cases after relapse (84.8% vs. 68.4%); return after default (84.8% vs. 57.6%); and re-treated cases after failure (84.8% vs. 66.7%) ($p < 0.01$). On the contrary, death rates were higher among PTB+ cases that returned after default (15.2%); re-treated cases for pervious failure (15.7%); and re-treated cases for previous relapse (11.2%) compared to new PTB+ cases (7%) ($p < 0.01$) (Table 2).

The defaulter rate among PTB+ cases that returned after defaulting treatment was high (26.6%), and such patients were more likely to default again compared to new PTB+ cases (7.8%) ($p < 0.01$). On the other hand, PTB+ patients re-treated for previous failure had higher treatment failure (7.8%) and were more likely to have

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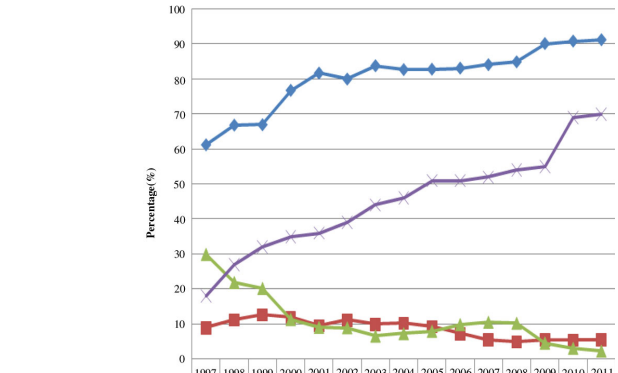

	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Treatment Sucess Rate(%)	61.3	66.9	67	76.8	81.7	80	83.8	82.7	82.8	83.1	84.1	84.9	90.1	90.8	91.2
Death Rate(%)	8.8	11.1	12.5	11.8	9.4	11	9.8	10.1	9.1	7.1	5.4	4.8	5.3	5.2	5.4
Defaulter Rate(%)	29.9	21.8	20.1	11.2	8.9	8.8	6.4	7.1	7.8	9.7	10.4	10.1	4.4	2.9	2.1
Population DOTS Coverage(%)	18	27	32	35	36	39	44	46	51	51	52	54	55	69	70

Fig. 2. Trends in TB treatment outcomes and population DOTS coverage, Arsi Zone, Central Ethiopia, 1997–2011.
Note: Trends in treatment success rate of PTB+ increased (X^2trend = 31.08, $p < 0.001$) with declined in death rate (X^2trend = 18.56, $p < 0.001$) and defaulter rate (X^2trend = 33.74, $p < 0.001$) over time. Trends in treatment success rate increased; death rate and defaulter rate declined with increase of DOTS population coverage (X^2trend = 22.243, $p < 0.001$).

treatment failure again compared to new PTB+ patients (0.4%) ($p < 0.01$) (Table 2).

Factors associated with unsuccessful TB treatment outcomes

Logistic regression analyses revealed that patients who are urban residents, older ones, re-treated cases, those co-infected with HIV, and those with no contact person were less likely to be treated successfully. In the final model, TB patients in the age group 25–49 (AOR, 0.26; 95% CI: 0.53–0.95) and above 50 years of age (AOR, 0.42; 95% CI: 0.33–0.60) were less likely to be treated successfully compared to the younger age groups. Moreover, re-treatment cases (AOR, 0.61; 0.41, 0.67) in comparison with new ones, and TB/HIV co-infected cases (AOR, 0.45; 95% CI: 0.31–0.53) in comparison with non-TB/HIV co-infected ones were less likely to be successfully treated (Table 3).

Treatment outcomes among PTB + cases by district

The overall treatment outcomes among PTB+ cases registered for treatment during the study period varied across the 25 districts of the zone ($X^2 = 317.35$, $p < 0.001$). The 15-year average TSR varied from 69.3 to

Table 2. Treatment outcomes of new smear-positive and re-treatment pulmonary TB cases of Arsi Zone, Central Ethiopia, 1997–2011

	Treatment outcomes														
	Cured		Treatment completed		Successfully treated		Died		Defaulted		Failed		Unsuccessfully treated		
Variable	No	%	No	%	No	%	No	%	No	%	No	%	No	%	Total
New (N)	9,085	68.6	2,138	16.2	11,223	84.8	933	7.0	1,026	7.8	55	0.4	2,014	15.2	13,237
Relapse (R)	468	54.0	125	14.4	593	68.4	97	11.2	167	19.3	10	1.2	274	31.6	867
Defaulter (D)	26	39.4	12	18.2	38	57.6	10	15.2	17	25.8	1	1.5	28	42.4	66
Failure (F)	29	56.9	5	9.8	34	66.7	8	15.7	5	9.8	4	7.8	17	33.3	51
Total	9,608	67.6	2,280	16.0	11,888	83.6	1,048	7.4	1,215	8.5	70	0.5	2,333	16.4	14,221
All re-treatment cases (R + D + F)*	523	53.2	142	14.4	665	67.6	115	11.7	189	19.2	15	1.5	319	32.4	984

Note: Total cases for each category are the sum of successfully treated and unsuccessfully treated of each category. *The sum of relapse (R), defaulter (D) and Failure (F).

92.5%. Defaulter rate ranged between 2.5 and 21.6%, whereas death rate spanned from 1.6 to 11.1%. It was also observed that districts with low TSR (69.3%) had high rates of default (16.9%), death (10.2%), and failure (3.6%) while those with high treatment success (more than 85%) had low rates of default and death. Moreover, high death rate was observed in districts that had high TB/HIV co-infection (Table 4).

Discussion

Expanding DOTS and improving its population coverage has increased TSR in PTB+ cases and has led to improved overall TB treatment outcomes in the study area. There are poor treatment outcomes among re-treatment cases compared to the new ones. Further, there are significant differences in TB treatment outcomes among the 25 districts of the zone. These findings might help the TB control programme managers and policy makers to look for an alternative strategy for addressing the inequality in TB treatment outcomes across different geographical settings in the country.

In this study, TB TSR increased significantly from 61.3 to 91.2% and parallel with the expansion of DOTS population coverage from 18 to 70% in 15 years. The upward trend in TSR was inversely proportional to the declining trend in defaulters which went from 29.9 to 2.1% and deaths from 12.5 to 5.4% over time. Such a result goes with findings by other studies that showed a similar trend of increase in treatment success and decline in death and default rate (5, 11, 20). Moreover, the increase in TSR during the last 3 years of the study period was significant and higher than the 85% target recommended by WHO.

The TSR increased by 31% through the improvement of population DOTS coverage by 52% in 15 years. This is higher than the 13.4% rise in TSR and the 44% increase in population DOTS coverage over 7 years in southern Ethiopia (11). It is also higher than the 15% increase in TSR over 14 years in Vietnam (20) and the 18% global average increase gained in a 10-year period (21). The increase in trend observed during the last 3 years of the present study is significant and could be attributed to the stepwise deployment of HEWs at community level. Since 2008, it has been possible to cover all *kebeles* through two female HEWs (19). The involvement of HEWs in a TB control programme might have improved access and played a significant role in the reduction of defaulter cases. This implies the importance of further decentralisation of DOTS service to the community level where there are resource constraints and limited access to health services to achieve MDG in TB control.

The overall 15-year average TSR among PTB+ patients was 83.6%. This is high compared to findings by previous studies which showed 29.5 and 28.3% in northwest Ethiopia (14, 22), 55.7% in western Ethiopia (23), and 77% in Tanzania (24). However, it is lower than the 88% success rate in China (25) and 89% in southern and northern Ethiopia (12, 26). The difference could be due to variation in the length of the study period and the sample size across different study areas. It could also be due to variation in the study setting. Majority of the patients in northern Ethiopia were from urban centres, and about 25% of them were HIV co-infected (14, 22)

Table 3. Factors associated with TB treatment success rate among pulmonary smear-positive TB patients registered during 1997–2011 in Arsi Zone, Central Ethiopia

		Treatment success categories			
Variables	Category	Not successfully treated	Successfully treated	COR (95% CI)	AOR (95% CI)
Patients' residence	Urban	794 (15.5)	4,325 (84.5)	1.00	1.00
	Rural	1,138 (12.5)	7,964 (87.5)	1.49 (1.40,1.58)**	1.13 (0.96,1.37)
Age	0–14 years	192 (9.9)	1,747 (90.1)	1.00	1.00
	15–24 years	421 (9.6)	3,968 (90.4)	1.06 (0.95,1.21)	1.14 (0.84,1.53)
	25–49 years	1006 (16.2)	5,201 (83.8)	0.59 (0.52,0.68)**	0.74 (0.53,0.95)**
	≥50 years	325 (19.3)	1,361 (81.7)	0.47 (0.44,0.56)**	0.42 (0.35,0.60)**
Sex	Male	1,060 (13.7)	6,674 (86.3)	1.00	1.00
	Female	895 (13.8)	5,592 (86.9)	1.05 (0.98,1.11)	1.10 (0.94,1.30)
Patient category	New	2,014 (15.2)	11,223 (84.8)	1.00	1.00
	Re-treatment	319 (32.4)	665 (67.6)	0.47 (0.43,0.56)**	0.61 (0.41,0.67)**
HIV status	HIV −ve	227 (6.8)	3,112 (93.2)	1.00	1.00
	HIV +ve	49 (17.1)	239 (82.9)	0.35 (0.34,0.47)**	0.45 (0.31,0.53)**
Having, contact person	Yes	1,770 (13.3)	11,537 (86.7)	1.00	1.00
	No	158 (17.0)	756 (83.0)	0.76 (0.68,0.85)**	0.87 (0.61,1.35)

Note: **Significant at $p < 0.001$.

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Table 4. Treatment outcomes of all pulmonary smear-positive (new plus re-treated cases) TB patients by districts in Arsi Zone, Central Ethiopia, 1997–2011

	Treatment outcomes for smear-positive pulmonary TB ($N = 14221$)					TB/HIV co-infection		
Districts	Total PTB+ *N*	Cured rate *N* (%)	Treatment success *N* (%)	Defaulter rate *N* (%)	Death rate *N* (%)	Failure rate *N* (%)	Number of tested *N*	HIV positive *N* (%)
Tiyo	131	58 (44.3)	113 (86.3)	12 (9.2)	4 (3.1)	2 (1.5)	42	4 (9.5)
Amigna	162	86 (52.4)	117 (72.2)	35 (21.6)	9 (5.6)	1 (0.6)	73	5 (6.8)
Aseko	373	259 (66.9)	310 (83.1)	41 (11.0)	21 (5.6)	1 (0.3)	138	2 (1.4)
Assala town	1,591	1,244 (76.7)	1,334 (83.8)	117 (7.4)	131 (8.2)	9 (0.6)	372	86 (23.1)
Ble Gezegar	256	128 (49.6)	214 (83.6)	24 (9.4)	18 (7.0)	0 (0.0)	58	4 (6.9)
Cholle	408	302 (72.8)	357 (87.5)	26 (6.4)	24 (5.9)	1 (0.2)	162	9 (5.6)
Digalutijo	970	715 (72.4)	829 (85.5)	55 (5.7)	79 (8.1)	7 (0.7)	247	16 (6.5)
Diksis	399	193 (47.9)	309 (77.9)	64 (16)	21 (5.3)	5 (1.3)	72	1 (1.4)
Dodota	1,425	911 (63.9)	1,149 (80.6)	108 (7.6)	158 (11.1)	5 (0.4)	180	36 (20)
Gololcha	444	303 (67.9)	401 (90.3)	35 (7.9)	7 (1.6)	1 (0.2)	222	5 (2.3)
Guna	466	238 (50.6)	344 (73.8)	80 (17.2)	40 (8.6)	2 (0.4)	98	2 (2)
Hetosa	840	570 (66.9)	688 (81.9)	84 (10.2)	66 (7.9)	2 (0.2)	218	20 (9.2)
H/Wabe	170	74 (43.3)	152 (89.4)	9 (5.3)	8 (4.7)	1 (0.6)	58	1 (1.7)
Jaju	961	730 (74.0)	821 (85.4)	59 (6.1)	77 (8.0)	4 (0.4)	253	11 (4.3)
L/Bilbilo	918	695 (74.1)	796 (86.7)	59 (6.4)	61 (6.6)	2 (0.2)	173	10 (5.8)
L/Hitosa	604	455 (73.6)	542 (89.7)	23 (3.8)	37 (6.1)	2 (0.3)	165	17 (10.3)
Merti	644	428 (65.5)	517 (80.3)	64 (9.9)	62 (9.6)	1 (0.2)	146	15 (10, 3)
Munesa	323	213 (65.1)	268 (83.0)	36 (11.1)	15 (4.6)	4 (1.2)	180	8 (4, 4)
Robe	996	657 (65.4)	821 (82.4)	92 (9.2)	78 (7.8)	5 (0.5)	169	14 (8.3)
Shirka	580	360 (61.6)	468 (80.7)	71 (12.1)	40 (6.9)	2 (0.3)	117	4 (3.4)
Sire	525	363 (68.5)	437 (83.2)	54 (10.3)	30 (5.7)	4 (0.8)	108	4 (3.7)
Sude	387	201 (51.3)	350 (90.4)	19 (4.9)	18 (4.7)	0 (0.0)	120	1 (0, 8)
Tena	166	98 (57.0)	115 (69.3)	28 (16.9)	17 (10.2)	6 (3.6)	68	5 (7.4)
Z/Dugda	320	233 (72.6)	296 (92.5)	8 (2.5)	15 (4.7)	1 (0.3)	101	3 (3)
Seru	167	91 (53.5)	140 (83.8)	13 (7.6)	12 (7.2)	2 (1.2)	87	5 (5.7)
Total	14,221	9,608 (67.5)	11,888 (83.6)	1,215 (8.5)	1,048 (7.4)	70 (0.5)	3,627	288 (7.9)

Note: Total cases include those under treatment success, defaulter, death and failure rates. L/Bilbilo: Lemu Bilbilo; L/Hitosa: Lode Hitosa; Z/Dugda: Zuway Dugda.

compared to the 7.9% in the current study (15). Obviously, TB/HIV co-infection is associated with poor treatment outcome (27). In addition, TB cases transferred out in large numbers in northern Ethiopia were included in the analysis, whereas the actual treatment outcome of this group is not known. Thus, their inclusion in the computation would influence the report on TSR (13, 14, 22).

The TSR among re-treatment cases is much lower than new PTB+ cases. This confirms previous studies where re-treatment cases were significantly associated with unsuccessful treatment outcome (11, 12, 28, 29). Moreover, the current study revealed that there is high death rate and failure rate among re-treatment cases compared to the new ones. WHO recommended a microbial culture and drug susceptibility test (DST) for all re-treatment TB cases and new PTB+ cases who failed to convert sputum examination results at the end of a second month of follow-up (30). However, due to limited access to culture and DST services in Ethiopia, the services were not provided for MDR suspected cases; hence, the extent of MDR among re-treatment cases was not known. Moreover, the high death and failure rate in this group in the current study might also be due to high prevalence of MDR-TB in the group (28, 29). Thus, the findings of the study might warrant further investigation to determine the extent of MDR-TB in Ethiopia. This could help in giving a quick response to the current global challenge of MDR-TB.

Reports from elsewhere revealed that the prevalence of MDR-TB was more than 10-fold among previously treated patients than untreated cases (28). Study results from northern Ethiopia also indicated that previous

anti-TB drug exposure had 6.4 times risk of developing MDR-TB compared to new TB cases (31). The prevalence of MDR-TB is estimated to be between 1.6 and 1.8% among new and between 12 and 18% among re-treatment cases in Ethiopia (8, 32). Establishing microbial culture and DST services at least at referral hospital to detect MDR-TB cases as early as possible and initiating appropriate treatment is an urgent issue to be addressed in Ethiopia.

The overall 8.6% 15-year average defaulter rate in the current study was lower than reports from elsewhere in and outside the country (10, 33–35). However, there are also other studies with lower rates than the rate in this study (22, 23) and the global WHO target of less than 5%. Patients returning for re-treatment after defaulting were much more likely to default again (26.6%) compared to new patients (7.0%). This finding is in line with previous reports where such patients were more likely to default again compared to new cases (11, 12). Poor adherence to anti-TB treatment due to defaulting and irregular treatment may lead to more severe illness, treatment failure, relapse, longer infection, drug resistance, and even death. Thus, defaulting and irregular intake of anti-TB drugs are a challenge and concern for the individual patient as well as for the community and they need to be addressed properly.

The average 15-year failure rate (0.5%) in the present study corroborates previous reports of 0.2, 0.5 and 0.3% failure rate in northwest and western Ethiopia (14, 22, 23). However, it is lower than the 1.2% rate among new PTB+ and the 6.4% among re-treatment cases in southern Ethiopia (11), the 3.2% among new PTB+ in north Ethiopia (12) and the 2.8% in China (25). The large number of transfer outs and defaulters in the present study might have exerted an influence on the failure rate as it might be higher if the transfer outs were evaluated and defaulters completed their treatment.

The present study indicated that TB patients in the age range of 25–49 and above 50 years, and those co-infected with HIV were independently associated with unsuccessful TB treatment outcome. This is supported by other studies (11, 12, 33, 35, 36) where similar results were observed. On the contrary, sex, area of residence, and patient category showed no significant association with unsuccessful TB treatment outcome, and this is inconsistent with findings from other studies as well (11, 13, 22, 35).

Findings from this study also illustrate a significant variation in treatment outcomes among patients across the 25 districts of the zone. The highest TSR (92.5%) with lowest defaulter (2.5%) and failure (0.3%) rates in Zuway Dugda district might indicate the role of effective TB treatment in the reduction of defaulter cases and drug-resistant strain. However, the lowest TSR (69.3%) with highest failure (3.6%), defaulter (17%), and death (10.2%) rates in Tena district might reflect the consequences of poor TB treatment (3, 4).

The high death rate observed among districts with high TB/HIV co-infection in the current study substantiates previous reports of high mortality rate among TB/HIV co-infected cases (3, 37). Overall, the variation in treatment success, defaulter, death, and failure rates across districts of the zone could be due to the real differences in DOTS performance and disparity in quality of TB control programme (11). Thus, the study warrants TB programme managers and policy makers to identify locality specific challenges to be addressed in order to universally achieve the global WHO recommended rate of 85% treatment success.

Although the study has established the usefulness of facility-based data analysis of a 15-year period, it has some limitations. This retrospective facility-based study lacks inclusion of patients' important variables such as educational level, knowledge about TB, duration of the treatment, distance from the treatment centre, family size, family support, medication side effect, and income that have been reported to have an association with TB treatment outcomes (12, 13, 33). Because of the inherent limitations of a retrospective study, incomplete data were excluded from the analysis and this might affect the results of this study. Moreover, counting all patients who were treated with a full course of anti-TB drugs but with missing records on their treatment outcomes as defaulters could introduce bias as these patients might have completed the treatment, died, or failed.

Conclusions

DOTS expansion and improving population DOTS coverage in Arsi has led to a significant increase in treatment success and a decrease in death and defaulter rates. However, there is a major variation in treatment outcomes across the 25 districts of the zone, so a district-specific intervention strategy needs to be considered. The low TSR among re-treatment cases might be due to a high rate of MDR-TB among this group, and the issue needs to be further investigated to identify the extent of the problem.

Authors' contributions

SDH was the principal investigator responsible for designing and conducting the study. Further, he was involved in analysing the data and writing up the manuscript. MD participated in preparing the design of the study and writing up the manuscript. DT was involved in data collection and analysis while BL took part in

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preparing the design, analysing the data, and writing up the manuscript. All authors read and approved the final manuscript.

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Conflict of interest and funding

The author(s) declare that they have no conflict of interests.

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Paper III

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III

Prevalence and Incidence of Smear-Positive Pulmonary Tuberculosis in the Hetosa District of Arsi Zone, Oromia Regional State of Central Ethiopia

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Abstract

Background: *The real burden of smear-positive (PTB+) and bacteriologically confirmed tuberculosis (BCTB) in Ethiopia is not known. Thus, the aim of this community-based study was to measure the prevalence and incidence of tuberculosis in the Hetosa District of Oromia Region, Ethiopia.*

Methods: *First, a population-based cross-sectional survey was conducted on a total of 33,073 individuals aged ≥ 15 years to determine the prevalence of PTB+ and BCTB cases. Then, in order to determine the incidence, a prospective follow-up was carried out on 32,800 individuals found to be either free from symptoms suggestive of TB (SSTB) during the baseline survey or had symptoms suggestive of TB but yielded negative bacteriological examination results. Each participants with cough of more than two weeks were provided spot and morning sputum samples for acid-fast bacilli sputum microscopy and culture.*

Results*: At the baseline survey, 43 BCTB cases were identified. Thirty six of these were both smear- and culture-positive while seven were only culture-positive. In the follow-up study, however, 76 BCTB cases were diagnosed and 70 of these were found to be both smear- and culture-positive while six were culture-positive only. The adjusted prevalence of PTB+ and BCTB in the study area was 109 and 132/100,000 persons, respectively. Moreover, the incidences of PTB+ and BCTB were 214 and 232/100,000 persons per year (py), respectively.The ratio of the passive to active case finding was 1:0.96 (45/43). For every TB case identified through the existing passive case diagnosis, there was an almost equal number (0.96) of undiagnosed infectious TB cases in the community. A family history of TB contact was independently associated with a high risk of TB (TB prevalence, AOR, 13; 95% CI: 6.55–15.33) and (TB incidence, aIRR 4.11, 95% CI: 2.18–7.77).*

Conclusions and recommendations: *The prevalence and incidence of PTB+ BCTB cases were high in the study area. For every case of PTB+ receiving treatment, there was an almost equal (0.96) number of undetected infectious bacteriologically confirmed TB case in the community. The high proportion of undetected infectious TB cases in the community could possibly be due to the sub-optimal performance of Directly Observed Treatment Short-course (DOTS).Family history of TB contact has substantaially increased the risk of developing the*

disease, and there is a need to improve ways of identifying TB cases and intensify mechanisms of tracing contacts among household members of PTB+ cases.

Keywords: pulmonary smear-positive TB, prevalence, incidence, Arsi Zone, Ethiopia

Introduction

Even though highly effective first-line short-course regimens that can cure about 90% of tuberculosis (TB) cases have been available for decades, the disease remains a major cause of morbidity and the second leading cause of death worldwide. Only in 2014,there were an estimated of 9.6 million TB incidents and 1.5 million deaths due to the disease worldwide [1-3].

In 1993, the World Health Organization (WHO) declared TB to be a global public health emergency and in 1994 formally launched the Directly Observed Treatment Short-course (DOTS) as a standard strategy to control the disease [4]. Since then significant progress has been made in reversing the incidence of TB and it was possible to reduce its prevalence by 41% worldwide [5]. However, in sub-Saharan Africa and other resource-constrained countries, the number of new TB cases reported is steadily increasing. Moreover, 80% of TB cases and 78% of global TB deaths occur in these countries, primarily due to the high prevalence of human immuno-deficiency virus (HIV), poor TB control efforts, social inequalities, drug resistance and inadequate access to TB care [4-6].

The incidence and prevalence of TB are among the valuable epidemiological indicators used to measure the impact of TB control efforts and assess the progress made towards the Millennium Development Goals (MDGs) [7-9]. A recent systematic review has shown that the current fixed value of the annual risk of TB infection derived using the Styblo rule in the estimation of TB incidence in the community is no longer valid in the era of the HIV epidemic due to the fact that the incidence of TB cases is fuelled by the powerful interaction between tuberculosis and HIV [9,10]. Hence, the true TB incidence and prevalence in the community could only be obtained through population-based surveys and prospective follow-up studies that measure the impact of TB control efforts in a particular country [8]. However, these types of data are lacking in developing countries including Ethiopia [11,12].

Moreover, estimating the incidence of TB is a challenge due to the fact that enrolling many people in a prospective follow-up study is difficult [8].As a result, two consecutive community-based prevalence surveys within a short time interval is an alternative option [8]. However, deriving TB incidence from prevalence surveys requires a good estimate of disease duration, which is difficult to obtain from such surveys. In general, the true TB incidence can be measured by either conducting a prospective follow-up study or by carrying out two consecutive prevalence surveys within a short time interval, and then estimating the number of new TB cases that occurs between the surveys [7-9].

According to the global TB report of 2015, the TB prevalence and incidence in Ethiopia were estimated at 190 (95% CI: 160–240) and 200 (95% CI: 160–240), respectively [1]. Moreover, the 2011 National TB Prevalence Survey and other reports from different parts of the country showed that the TB prevalence ranged between 30 and 213.4 per 100,000 population [12-18]. However, the real burden of smear-positive pulmonary TB and bacteriologically confirmed TB cases in Arsi Zone, in general, and Hetosa District, in particular, was not known. Thus, the aim of this study was to measure the prevalence of bacteriologically confirmed pulmonary TB at the baseline survey, and then to investigate the incidence of TB through a prospective follow-up study in the Hetosa District of Arsi Zone, Central Ethiopia.

Methods

Study setting

The Hetosa District is one of the 25 districts of Arsi Zone, Oromia Regional State of Central Ethiopia. The district is typical of the zone in terms of population density, socio–cultural and economic state, and demographic conditions. Therefore, it is expected that the results could be generalized to the whole zone. Based on the 2007 Census Projection, the district has an estimated population of 178,229 living in one urban and 23 rural *kebele*s (the smallest administrative unit in the government structure) [19]. Since 2010, each *kebele* in the district has been further divided into six sub-*kebele*s in the rural areas and 10 sub-*kebele*s in the urban centres. The sub-*kebele*s are known as *garee*s. The *garee*s of the district total to148 (138 from rural and 10 from urban *kebele*s) [20]. This study was carried out in 49 randomly selected *garee*s (clusters).

Study design and population

A population-based cross-sectional survey using multi-stage cluster sampling method was used to estimate the prevalence of smear-positive TB (PTB+) and bacteriologically confirmed TB (BCTB) cases at baseline. Next, a prospective follow-up study design was employed to estimate the incidence of the disease. In the estimation of PTB+ and BCTB incidence, individuals who were free of a persistent cough for more than two weeks, fever, loss of appetite, weight loss, blood-stained sputum and chest pain or difficulty in breathing [symptoms suggestive of TB (SSPTB)] at baseline, and those who had SSPTB at baseline but later showed negative result in a bacteriological test, were adopted as a cohort for the prospective follow-up study. The study was carried out from July 2013 to June 2014.

The source population for the study were adult individuals aged ≥15 years and permanently living in the district. Eligibility criteria were age ≥15 years, willingness to provide written consent to participate in the study, and a permanent residence for at least 15 days in the selected house prior to the start of the study. Additionally, participants had to be individuals with SSPTB.

Sample size and sampling techniques

For economic and practical reasons, and because it is typical of the whole of Arsi Zone, the Hetosa District was purposefully selected from the 25 districts in the zone. All the 23 rural and one urban kebeles of the district were included in the study. The number of eligible population in the urban kebele was 5,903, and the population in the 23 rural kebeles was 27,170. Moreover, 18 of the rural kebeles have higher population density compared to the remaining five. Each of the former kebele has about double the population size of each of the latter. The number of clusters (garees) allocated to each urban and rural kebele was proportional to its population size.

Consequently, a stratified multi-stage random sampling procedure was used to select two garees from each of the 18 rural kebeles high population density, and one garee from each of the remaining five rural kebeles. Moreover, based on the urban population of 5,903 and rural population of 27,170 that fulfilled the eligibility criteria, eight garees from the urban and 41 from the rural kebeles were included in the study [20]. An alphabetically arranged list of garees in each kebele along with their population size was obtained from the district

authorities. Subsequently, garees were randomly selected from the list and included in the study. All individuals aged ≥15 years residing in the selected garees were included in the study.

The prevalence of PTB+ used in the sample size calculation was 382 per 100,000 based on the assumption that has been used in the 2011 national prevalence survey of the adult population aged ≥15 years [21], and an estimated 210/100,000 in 2012 for Ethiopia by the WHO [22]. Consequently, we calculated the sample size using a prevalence of 382 per 100,000, a relative precision of 0.25, an expected participation rate of 90% and a design effect of 2, a sample size of 33,448 people from 49 clusters. However, in the house-to-house enumeration held during the pre-survey of all the 49 selected *garee*s, 34,707 adults aged ≥ 15 years were identified. As the number was very similar to that of the calculated sample size, we enrolled all of them in the study.

Data collection procedures

The aims of the study and the procedures for data collection were discussed with zonal, district and *kebele* leaders. The District TB Coordinator selected 24 nurses and 24 health extension workers (HEWs) for data collection, five laboratory technicians for sputum sample collection and 10 health officers for supervision. A health extension worker (HEW) is a female community health worker trained for one-year and deployed at a *kebele* with the responsibility of providing essential health services to ensure equitable access to health care, prevent major communicable diseases and promote health in the community[23]. Altogether, a total of 48 data collectors, five laboratory technicians and 10 supervisors were trained on TB screening techniques and on how to collect and transport sputum specimens. The baseline survey to determine the prevalence of PTB+ and BCTB was conducted in May to June 2013. Subsequently, the prospective follow-up study to determine the incidence of PTB+ and BCTB was carried out between July 2013 and June 2014. Individuals who met the eligibility criteria, and were willing to provide written consent to participate in the study were included.

Study participants with SSPTB were identified as presumptive TB cases and were interviewed about their age, sex, history of contact with known TB patients and any current or previous TB treatment both at the baseline survey and prospective follow-up study. Participants with any SSPTB were requested to submit two sputum samples, one on the spot and the other in the

morning of the following day. Upon receipt from the participants, the specimens were immediately put in sterile flacon tubes and placed in a cold box at 4°C and transported on the same day to the Adama Regional Research Centre Laboratory.

The following day each smear was fixed, air-dried and stained using the standard Ziehl-Neelsen (ZN) methods [24] and examined by experienced laboratory technicians for the presence of acid-fast bacilli (AFB). Positive results were quantified using the International Union against Tuberculosis and Lung Disease (IUATLD) standards [25]. A senior laboratory technologist blinded to the first test results re-examined all the smear-positive and 10% of the smear-negative slides. However, no discordant test results were observed between the two examinations. Moreover, sputum cultures using Lowenstein-Jensen (LJ) medium were commenced within a maximum of two days from the receipt of the sputum. In the event the diagnostic test did not commence on the 2nd day following specimen collection, the sputum sample was stored at -20°C in the same laboratory until tests were undertaken.

The data collectors also checked TB patient registration units in the study area to verify that those who reported they were on anti-TB treatments at the time of the study were actually on medication, and if there was any patient who was on anti-TB medication but did not report it during the survey period. However, no mismatch was identified.

Pulmonary smear-positive TB (PTB+) is defined as a patient found to be positive for AFB in both spot and morning sputum samples examined using direct microscopy or a patient found to be smear-positive in either spot or morning sputum examinations for AFB and culture-positive. Further, bacteriologically confirmed TB (BCTB) cases are individuals with smear- and/or culture-positive results. Types of TB were defined based on the 2011 WHO Tuberculosis Prevalence Survey Handbook [26] as follows:

New case not on treatment: A patient who has never received TB treatment for more than a month and who is not being treated currently with any anti-TB drugs.
New case on treatment: A patient who is currently being treated with anti-TB drugs, but has previously not received any anti-TB treatment for more than a month.

Previously treated case not on treatment: A patient who has previously received treatment for TB for more than a month, but who is currently not receiving any treatment with anti-TB drugs.
Previously treated case on treatment: A patient who has previously received treatment for TB for more than a month and who is currently being treated with anti-TB drugs.

The sputum smear-positive results were communicated through both written and telephone reports to TB focal persons at health centres in the study sites. The diagnosed TB cases started anti-TB treatment according to the national TB guidelines [24], with a culture performed on morning specimens using Lowenstein-Jensen (LJ) medium. The results were considered to be negative if no colonies were identified after eight weeks of incubation. Positive results from the LJ cultures were confirmed by testing for the presence of AFB through microscopic examination using the Ziehl-Neelsen method.

In the follow-up study, a total of 32,800 individuals who were free from SSPTB at baseline study and those with SSPTB but negative bacteriological results during the same survey were followed up for 12 months (July 2013 to June 2014) so as to estimate the incidence of PTB+ and BCTB cases. At intervals of six months, both at the end of the sixth and the 12th months from the baseline study, the same data collectors revisited all households that had been visited at the baseline and interviewed each person aged ≥15 years. The same data collection procedure, sputum sample collection, laboratory testing procedures and questionnaire were used in the prospective follow-up study. To ensure the data quality, the principal investigator and supervisors closely monitored the data collection process.

Data entry and analysis
All data collected using the standardized and pre-tested questionnaire were coded and double-entered into Epi-info version 7 statistical software. The data were checked against the original questionnaires for missing variables, and errors were corrected by referring to the original questionnaires. Data analyses were performed based on the method recommended by the WHO Tuberculosis Prevalence Survey Handbook for the estimation of PTB+ and BCTB prevalence [26]. In the initial model of analysis, the crude PTB+ and BCTB prevalence was estimated without taking into account the sample cluster survey design effect.

Nonetheless, in the final model, a complete analysis with an inverse probability weighting was carried out using robust standard errors to account for the sample cluster survey design effect, and the adjusted estimated prevalence of both PTB+ and BCTB were computed and reported [26]. Data analysis was carried out using STATA (v12.1, Stata Corporation, College Station, TX, USA).

Furthermore, in the analysis of TB incidence, a persons per year observation (pyo) was used as a denominator where person-time at risk of TB began in June 2013 when eligible individuals started participating in the study. Enrolment ended when participants were found to be AFB and/or culture-positive and were censored in June 2014. However, as the exact time of contribution of those who dropped out in the course of the study due to out-migration or death was not known, we excluded 185 participants (17 deaths, 47 refusal cases and 119 out-migrants) from the analysis to avoid bias due to ambiguity surrounding the time of their contribution. In order to avoid an under estimation of TB incidence, the principal investigator checked all health facilities in the study area to verify whether any TB cases were diagnosed and registered during the 12 months of the follow-up study, but none was found.

The prevalence and incidence of PTB+ and BCTB were taken as the dependent variables whereas age, sex, area of residence and family history of TB contact were the independent variables. The independent and dependent variables were further categorized into groups for analysis. A Poisson regression analysis was carried out in the analysis of TB incidence. The estimated incidence rate ratios (IRRs) and adjusted odds ratio (AOR) at 95% confidence intervals (CI) and *P*-values of less than 0.05 were used to assess the strength of association with PTB+ and BCTB cases as the outcome.

Ethical considerations

The study protocol was reviewed and approved by both the Regional Committee for Medical and Health Research Ethics in Western Norway (REK Vest) and the Institutional Review Board Committee at the Oromia Health Bureau, Ethiopia. All participants were informed that taking part in the study was based fully on their willingness, and that they had the right to quit at any time from the study. Before any interview started, in the prevalence survey and in the subsequent follow-up study written consents were obtained from all participants aged ≥18

years and from parents/guardians if participants were <18 years of age. Data on individuals were analysed and anonymously reported. Immediate referrals were arranged for participants found to be smear or culture-positive, and all started anti-TB treatment at health centres close to them. The principal investigator also confirmed that all patients started treatment.

Results

Survey population

A total of 63,312 individuals were enumerated during the pre-survey census (Figure 1). Of these, 34,707 were eligible and thus participated in the prevalence survey. Of participated, 33,073 (95.3%) were screened for SSPTB. The average number of eligible individuals who participated from each cluster was 674.9. The mean age of the screened individuals was 33.3 years (standard deviation; SD 16.2) and the median age was 30.3 years. The overall response rate was 95.3%, with 95.8% for females and 94.8% for males. The overall participation rate was over the 90% expected in the study design. However, a higher participation rate among rural clusters (97.6%) was seen compared to the urban ones (85.9%). Out of the total of 34,707 eligible individuals, 1,634 (4.7%) did not participate in the prevalence survey. Of the latter, 1,489 (91.1%) were not at home, while 145 (8.9%) were not willing to participate in the survey (Table 1 and Figure 1).

Screening and sputum submission

A total of 33,073 eligible individuals were screened for SSPTB in the prevalence survey. Of these, 27,173 (82%) were rural residents and 16,907 (51.1%) were males. Moreover, a total of 32,800 individuals were enrolled for the follow-up study (Table 1). Of these, 31,802 were free of SSPTB at the baseline survey, while 998 showed SSPTB but yielded a bacteriologically negative result. A total of 1,041 and 1,468 individuals at the baseline survey and during the follow-up study, respectively, were reported to have SSPTB. These provided two sputum samples for bacteriological examination (AFB microscopic examination and culture). Of the former group, 258 (24.8%) individuals reported previous history of TB treatment, but were not on anti-TB treatment at the time of the survey. However, 45 known PTB+ cases were diagnosed through passive TB case findings and had been on anti-TB treatment at the time of

the study. These also provided two sputum samples for bacteriological examination. Figure 1 summarizes the screening and subsequent bacteriological examination results.

TB cases identified

A total of 1,041 individuals with SSPTB and 45 PTB+ cases known to be on anti-TB treatment at the baseline survey provided spot and morning sputum samples for bacteriological examination. Of the 1,041 presumptive TB cases, 43 were found to have bacteriologically confirmed TB (culture and/or smear positive) whereas none of the 45 individuals on anti-TB treatment showed a positive result (Figure 1).

The mean (SD) age of the diagnosed TB cases was 30 (10.3) years and the median was 30.8 years. Of the 43 bacteriologically confirmed TB cases, 36 were both smear- and culture-positive while seven were smear-negative but culture-positive. From the 36 smear-positive TB cases, 12 (33.3%) had a family history of TB contacts (Table 2).

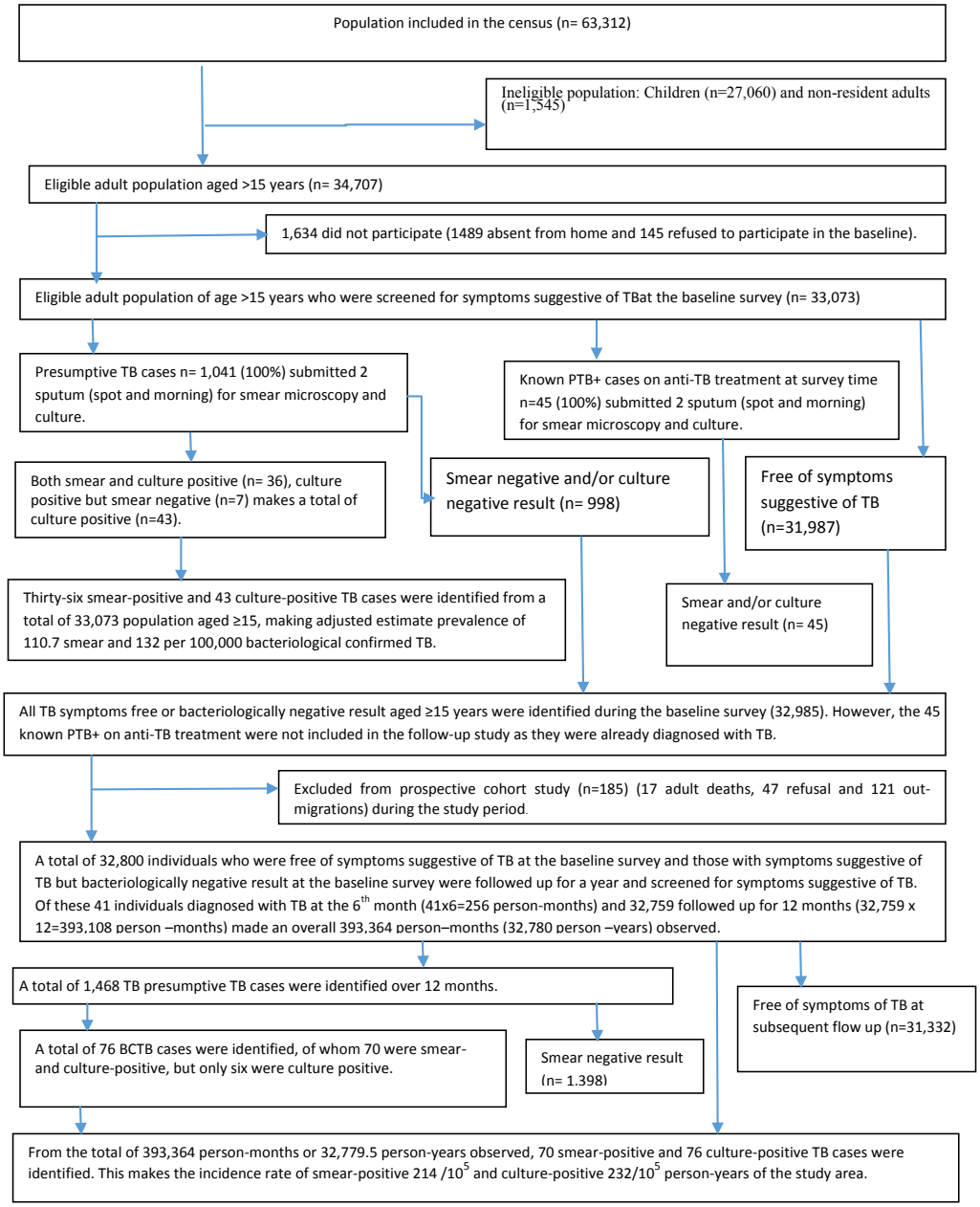

Figure 1: Study flow chart showing the study participants, screening procedure, sputum smear and culture results, Hetosa District, Arsi Zone of Oromia Region, Central Ethiopia, 2015.

Table 1: Participation of eligible individuals in the prevalence survey, Hetosa District of the Arsi Zone, Central Ethiopia

Variable	No. of eligible individuals identified through census and expected to participate	No. of eligible individuals who participated in the survey and were screened	Proportion of screened participants form the total eligible population
Sex			
Male	17,834	16,907	94.8
Female	16,873	16,166	95.8
Total	34,707	33,073	95.2
Age			
15-24	12,115	11,691	96.5
25-34	8,331	7,889	94.7
35-44	5,959	5,697	95.6
≥45	8,302	7,796	93.9
Mean age	33.3 (SD 16)		
Total	34,707	33,073	95.2
Stratum			
Urban	27,838	27,170	97.6
Rural	6,869	5,903	85.9
Total	34,707	33,073	95.2
PTB+ identified through passive case findings			
Male	28	Male to Female ratio	
Female	17		
Total	45	1: 0.61	
Number of BCTC identified by the survey			
Male	19	Male to Female ratio	
Female	24		
Total	43	0.79:1	

NB: PTB+ is smear-positive pulmonary Tuberculosis and BCTB is bacteriologically confirmed Tuberculosis cases.

Furthermore, 32,800 individuals were enrolled in the follow-up study. Of these, 32,759 were followed for 12 months, making 393,108 person-months of observation, and 41 were diagnosed with TB at the end of the sixth month making 246 person-months of observation. Overall, a total of 393,354 person-months or 32,779.5 person-years (py) were observed. Of the total of 393,354 person-months or 32,779.5 py observed, 76 bacteriologically confirmed TB cases were identified (41 at the end of the sixth month and 35 at the end of the 12th

month). Of these, 70 were smear- and culture-positive while the remaining six were smear-negative but culture-positive (Figure 1 and Table 3).

Prevalence

The adjusted prevalence estimate of PTB+ individuals among adults aged ≥15 years was 109 (95% CI: 67.2–150.5) whereas that of BCTB was 132 (95% CI: 83.0–176.2) per 100,000 population. Even so, the adjusted prevalence of BCTB was higher among females (148.5) (95% CI: 81.1–210.4) than males (112.2) (95% CI: 55.8–170.2) but had no statistical difference (Table 2).

In the multivariate logistic regression model, age and family history of TB contacts were independently associated with high rates of PTB+ and BCTB cases. Compared to individuals in the age group from 15–24 years, those in the age group from 25–34 years were 3.4 times more likely to have TB [AOR: 3.4 (95% CI: 1.4–8.6)]. Those in the age group of 35–44 years were 4.2 times more likely [AOR: 4.2 (95% CI: 1.7–10.2)] while those ≥45 years were 2.7 times more likely to have the disease [AOR: 2.7 (95% CI: 1.1–6.7)]. The prevalence of TB therefore increased with age up to 44 years but declined from 45 years onward. Presumptive TB for those who had a family history of contact with TB patients was 13 times more likely than those without such a history [OR = 13.0, (6.5–25.3)].

The active and passive TB case findings of the study area were compared using the number of cases identified by each method. Forty-three BCTB cases were identified through current active TB case findings while 45 PTB+ cases on anti-TB treatment at the time of the survey were detected through passive case findings. Of the 45 PTB+ cases, 28 were males while 17 were females making a ratio of 1:0.61 (28/17). The male to female ratio for those identified through active case findings was 0.79:1 (19/24). The ratio of passive to active case findings was 1:0.96 (45/43) (Table 1).

Table 2: Prevalence of smear-positive and bacteriologically-confirmed pulmonary TB among population aged ≥15 years, Hetosa District of Arsi Zone, Central Ethiopi

Category	Number of participants	Smear-positive Pulmonary Tuberculosis (PTB+)			Bacteriologically Confirmed Pulmonary Tuberculosis (BCTB)			
		Number of PTB+	Crude Prevalence estimate of PTB+ (95% CI)/100,000	Adjusted* prevalence estimate of PTB+ (95% CI)/100,000	Number of BCTB	Crude Prevalence Estimate of BCTB (95% CI)/100,000	Adjusted* Prevalence estimated of BCTB (95% CI)/100,000	Adjusted Odds Ratio
sex								
Male	16,907	16	89 (48.3-141.0)	89 (42.2- 147.1)	19	112 (62.0-163)	112.2 (55.8-170.2.)	1.00
Female	16,166	20	124 (69.5-177.9)	124 (63.4-184.0)	24	149 (89.1-215.0)	148.5 (81.9-210.4)	1.3 (0.71-2.43)
Age								
15-24	11,691	5	43 (5.3-80.3)	43 (4.1-81.3)	8	68 (26.3-92.2)	68 (19.1-99.4)	1.00
25-34	7,889	12	152 (66.1-238.1)	152 (60.0-244.2)	13	165 (75.2-254.0)	165 (68.0-261.2)	3.4 (1.38-8.61)
35-44	5,697	11	193 (79.1-307.1)	193 (73.0 -313.2)	11	193 (79.1-307.2)	193 (72.0-314.4)	4.2 (1.71-10.20)
≥45	7,796	8	103 (31.5-237.7)	103 (25.4-233.8)	11	141 (57.8-224.4)	141 (50.6-231.6)	2.76 (1.14-6.72)
Residence								
Urban	5,903	5	85 (10.5-159)	85 (4.4-165.1)	9	153 (104.2-254.0)	153 (97.0-261.2)	1,23 (0.59-2.57)
Rural	27,170	31	114 (73.9-154.2)	114 (67.5-160.3)	34	125 (97.3-224.4)	125 (90.1-231.6)	1.00
History of TB contact								
NO	31,170	24	77 (42.0-107.7)	77 (36.0-113.8)	31	98 (65.2-133.8)	98 (58.0-141.0)	1.00
Yes	1,867	12	643 (280.2-1005.2)	894 (274.1-1011.3)	12	643 (280.2-1005.2)	643 (273.0-1012.4)	13.0 (6.55- 15.33)
Total	33,037	36	109 (73.3-144.3)	109 (67.2 – 150.5)	43	130.2 (91.2-169)	130.2 (83-176.2)	

Incidence of smear-positive TB

From the total of 393,354 person-months (32,779.5 py) observed, 76 BCTB cases were identified. Of these, 70 were both smear- and culture-positive, while six were smear-negative but culture-positive. The incidence rate of PTB+ among adult individuals aged ≥15 years was 214 (95% CI: 163.5–263.5) whereas that of BCTB was 232 (95% CI: 179.7–283.9)/100,000 py in the study area (Table 3). There were 17 adult deaths, 47 refusals and 121 out-migrations during the study period and these were excluded from the analysis. Moreover, because the TB status of 1,634 individuals who did not participate in the baseline survey was not known, they were also excluded from the follow-up study (Figure 1).

The incidence of PTB+ cases among males was 215 (95% CI: 144.8–285.3) whereas it was 212 (95% CI: 140.7–283.2) among females per 100,000 py. Likewise, the incidence of PTB+ per 100,000 py among urban residents was 222 (95% CI: 101.5–343.4) while it was 212 (95% CI: 156.7–266.5) among rural people. However, the difference in the incidence rates among males and females (adjusted incidence rate ratio) [aIRR, 1.22 (95% CI: 0.78–1.93], and among urban and rural dwellers [aIRR, 1.23 (95% CI: 0.71–2.14)] was not statistically significant (Table 3).

In the multivariate Poisson regression model, the age and history of TB contact were independently associated with a high risk of TB. Presumptive TB cases in the age group from 35–44 years were 2.4 times [aIRR, 2.40 (95% CI: 1.18–4.55)] more likely to have TB compared to those in the younger age group from 15–24 years. Compared to the same age group, those aged ≥45 were 2.7 times [aIRR 2.66 (95% CI: 1.44–4.91)] more likely to develop the disease. Presumptive TB cases who were either free of SSPTB or showed negative bacteriological examination results at the baseline survey but had history of contact with TB patients in the family were four times more likely to have TB than those with no history of such contact [aIRR, 4.11 (95% CI: 2.18–7.77)] (Table 3).

Table 3: Study population, smear-positive TB cases identified over 12 months and incidence rate per 100,000 persons per year, Hetosa District, Arsi Zone of Oromia Region, Central Ethiopia

Category	Person-year	Smear-positive Pulmonary Tuberculosis (PTB+) (n=70)			Bacteriologically Confirmed Pulmonary Tuberculosis (BCTB)(n=76)		
		Number of diagnosed PTB+ cases	Incidence rate per 100,000 person-year (95% CI)	Adjusted Incidence Rate Ratio (aIRR) (95% CI)	Number of BCTB	Incidence rate per 100,000 person-year (95% CI)	Adjusted Incidence Rate Ratio (aIRR) (95% CI)
sex							
Male	16,741	36	215 (144.8-285.3)	1.00	38	227 (156.7-299.2)	1.00
Female	16,038.5	34	212 (140.7-283.2)	1.22 (0.78 -1.93)	38	237 (161.6-312.3)	1.26 (0.80-1.97)
Age							
15-24	11,631.5	16	138 (70.2-205)	1.00	19	163(64.1-236.8)	1.00
25-34	7,865	8	102 (31.2-172.2)	1.30 (0.64-2.67)	9	114 (39.7-189.2)	1.51(0.73-3.15)
35-44	5,514	20	363 (203.7-521.7)	2.40 (1.18-4.55)	21	381(218.0-543.7)	2.76 (1.38-5.52)
≥45	7,769	26	335 (206.0-463.3)	1.66 (1.44-4.91)	27	348 (216.4-478.6)	3.05 (1.63-5.71)
Residence							
Urban	5,843.5	13	222 (101.5-343.4)	1.00	16	274(139.6-408.0)	1.00
Rural	26,936	57	212 (156.7-266.5)	1.23 (0.71-2.14)	60	223(166.4-279.10	1.25 (0.72-2.16)
History of TB contact							
NO	31,138	50	161 (116.1-205.1)	1.00	56	180(132.7-217.6)	1.00
Yes	1,641.5	20	1218 (686.4-1752.4)	4.11 (2.18-7.77)	20	1218(686.4-1752.4)	5.11 (2.63-9.96)
Total	32,779.5	70	214 (163.5-263.5)		76	232(179.7-283.9)	

Discussion

This population-based study identified a high prevalence and incidence of PTB+ and BCTB among individuals aged ≥15 in Hetosa District of Arsi Zone. For every TB case of PTB+ on treatment, there was an almost equal number (0.96) of undetected bacteriologically confirmed infectious TB cases in the community. The overall crude prevalence point estimate of PTB+ and BCTB cases was very similar to the inverse probability weighting prevalence point estimate using robust standard errors to account for the cluster survey sample design effect. Even so, there was a difference in precision with a wide confidence interval for the adjusted prevalence point estimate. The adjusted prevalence estimate of PTB+ and BCTB cases in the study area was 109 (95% CI: 67.2–150.5) and 130.2 (95% CI: 83.0–176.2)/100,000 population, respectively. In the follow-up study, the incidence of PTB+ and BCTB was 214 (95% CI: 163.3–263.5) and 232 (95% CI: 179.7–283.9)/100,000 py.

The 130.2/100,000 adjusted prevalence estimate of BCTB cases identified in this study is higher than the 34/100,000 reported from China and the 76/100,000 from Southwest Ethiopia [12]. Nonetheless, it is lower than the 169/100,000 report from Northern Ethiopia [27] and the 278/100,000 from the Lao PDR [28].

Moreover, the 109/100,000 adjusted prevalence of PTB+ cases in this study is similar to the 108/100,000 report of the national estimate [29]. Conversely, it is higher than previous reports that ranged from 30 to 80/100,000 population in different parts of the country [12,14,30], the 90/100,000 from Eritrea [31] and the 95/100,000 from Bangladesh [32]. Still, it is lower than the145/100,000 population reported from Vietnam [33], and the 169/100,000 population reported from Northern Ethiopia [27] and India [34].

The difference in the prevalence of BCTB and PTB+ TB cases across different geographic settings might be due to differences in the populations studied, the timing of the study or differences in the sampling, data collection and screening methods used across the different studies. For example, in some studies [12,14,30] the heads of households were interviewed to give testimony about the TB symptomatic cases of all family members in the household. However, the heads of households may not have sufficient information about all individual

members while others interviewed all members of a household to screen presumptive TB cases [13,29].

Some community-based TB prevalence studies used clinical diagnoses and chest x-rays before taking sputum for screening [29,35,36] while others, including the current study, used TB symptom-screening questionnaires [12-14,36] to identify the cases. Nonetheless, the chances of detecting TB cases among non-symptomatic individuals increased by 20–50% when a combination of a TB symptom-screening questionnaire and a clinical diagnosis with a chest x-ray was employed, compared to using a TB symptom-screening questionnaire alone without a chest x-ray [7,28,29,35,37]. Hence, the adjusted estimate of BCTB prevalence in this study might be underestimated due to the fact that the chest x-ray screening method was not used to identify non-symptomatic TB cases.

The age group in the survey also varied across different studies. Some covered all age groups ≥15 years [29,30,32,38] while others included those aged >14 years [14] and still others those aged 14 years [12,13]. Consequently, a comparison of TB prevalence rates among studies within a country or elsewhere should be taken with caution.

The high prevalence of BCTB cases among younger age groups in this study is in agreement with a previous report [29], whereas the high prevalence of TB among the younger population may suggest ongoing TB transmission in the community. The prevalence of TB increased with age among the younger age groups up to the age of 45 years. However, a high TB incidence rate was observed among the older age group. This might be due to the high number of infectious TB cases identified at baseline, which could reduce the ongoing TB transmission among the general population, while the high TB incidence among the elderly is probably indicative of a latent TB reactivation among the older age group [13,14,29,39]. Nevertheless, further study is required to fully understand why the observed high TB prevalence among younger individuals also corresponded to a high incidence in the older age group.

In this study, 43 BCTB cases were identified through active TB case findings at the baseline survey while 45 PTB+ cases were identified through existing passive TB case findings. Thus, the ratio of PTB+ cases being treated at the time of the survey to newly detected BCTB cases

was 1:0.96 suggesting that for every PTB+ case receiving treatment during the survey, there was an almost equal number (0.96) of cases of BCTB existing in the community. This indicated that there was a very high proportion of undiagnosed infectious TB cases present in the community.

In Southern Ethiopia, there were two cases [14] while in South Africa there were 4.5 cases [40] of passive detection for every TB case identified through active case findings. In Northern Ethiopia, the ratios of passive to active TB case findings were 2.5:1 [30] and 2:1 [13]. This implies that there is a high number of undiagnosed infectious TB cases in the present study area compared to reports by previous studies. The high proportion of undetected infectious TB cases in the community might be due to the sub-optimal DOTS performance in identifying 70% of infectious TB cases and attaining the global target of 85% cure rate in Ethiopia [41-43].

Moreover, the difference in the number of undetected infectious TB cases across different geographic settings might be attributed to variation in DOTS performance, DOTS service coverage and the quality of DOTS services across various study areas. It could also be attributed to the difference in DOTS service uptake that might result from differences in public awareness about TB. Consequently, the decentralization and strengthening of the community in TB care could help to pick up undetected infectious TB cases in Arsi Zone.

The male-to-female ratio among PTB+ cases identified through existing passive TB case findings was 1:0.61 (28/17) whereas the ratio among BCTB cases identified by the current active TB case finding was 0.79:1 (19/24). This may indicate a lower rate of passive case findings among females compared to males. The lower passive and higher active TB case findings among women in this study is in agreement with reports from Southern [14] and Northern Ethiopia [13,27], Bangladesh [44] and India [34] where more women were identified through active TB case findings. The lower passive TB case findings among females might be due to poor access to health services, and as shown in a study conducted in South Africa, women are less likely to be asked for a sputum sample when they appear at health facilities[45]. Moreover, their economic dependence and low health-care seeking behaviour possibly hindered women from visiting health institutions to obtain TB care services. Barriers

to accessing health services among TB patients and a failure to detect women with TB through the routine TB control programme warrant further inquiry.

As expected, history of TB contact increased the risk of having active TB. A recent systematic review and previous reports have shown that history of TB contact was associated with a high risk of TB [30,46-48]. Findings by the current study are in line with those of a systematic review and large epidemiological surveys that have established the association between history of TB contact and higher risk of TB [47,49,50]. Therefore, contact-tracing efforts should target households with members who are PTB+ so as to capture the undetected infectious TB cases in the community.

In this study, the high prevalence of BCTB cases in urban areas confirms previous reports of high TB prevalence in urban settings [16,51,52]. In contrast, the national prevalence TB survey reported higher TB prevalence among dwellers in the rural areas [29].This is due to the inclusion in the national prevalence survey of pastoralists in the rural population where the highest prevalence ratio of 170/100,000 was observed [21] as opposed to the current study. The pastoralist population may have poor access to TB care, as well as low awareness and health-seeking behaviour which might have resulted in them having a high burden of undiagnosed TB cases and eventually elevating the prevalence of TB among the rural population in the national prevalence survey. The higher prevalence of BCTB cases among urban settings compared to rural areas in the current study may be due to the overcrowded living conditions and dichotomy of higher HIV prevalence in the urban areas of the country.

The 214/100,000 py incidence rate of PTB+ cases in this study is similar to the 212/100,000 py reported from South Africa [53]. However, it is higher than the 197/100,000 py from Guinea-Bissau [54] and the 207/100,000 py from Southern Ethiopia [15]. Nonetheless, it is lower than the 311/100,000 py reported from Northern Ethiopia [11]. The high incidence of TB in the present study might be an indication of the ongoing transmission of the disease that might result from a sub-optimal DOTS performance in the interruption of TB transmission. For instance, according to previous reports, there was a low rate of PTB+case detection rate (37.7%)[41] and a low cure rate (66.9%)[42] and a high prevalence of drug resistance TB [55] in the study area. Hence, the low PTB+ case detection rate, cure rates and high drug resistance

TB reported from the study area, combined with the high prevalence and incidence rates identified by the current study, may confirm the sub-optimal performance of DOTS in curbing the active transmission of TB. Therefore, the involvement of health extension workers in educating the community on TB as well as accelerating referral of presumptive TB cases may improve the possibility of capturing undiagnosed infectious TB cases in the community.

Information on the prevalence and incidence of TB is a valuable epidemiological indicator to help assess the impact of national and international TB control efforts. Nevertheless, community-based data on BCTB prevalence and incidence are lacking in developing countries including Ethiopia. As a result, the findings of this study with regard to the prevalence and incidence rates of BCTB cases are among the very few population-based studies in resource-poor settings.

In this study, efforts were made to maintain the quality of the study, and rigorous training was given for data collectors and laboratory technicians. The study population was monitored to identify deaths and migrations during the prospective follow-up study to provide an accurate time contribution in the denominator to compute the incidence rate. Moreover, we have used very sensitive standardized and pre-tested questionnaires to screen presumptive TB cases experienced and qualified laboratory technicians to carry out smear microscopy and sputum culture. Following that, a senior laboratory technologist who was blinded to the results of the first test results re-examined all the smear-positive, and 10% of the smear-negative slides, to validate the quality of laboratory results.

Additionally, the estimated design effect we have used in this study was 2 whereas the actual calculated design effect from the current study data was 1.3 there by indicating that the sample size of our study was adequate and is representative of the study population of the district. Likewise, in order to obtain the adjusted precision of PTB+ and BCTB prevalence of the study, the design effect for the cluster sample survey was taken into account during the analysis.

On the other hand, although our study was among the very few attempts to detect a community-based TB incidence and may contribute valuable information to the TB control

programme in Ethiopia, it has some limitations. First, SSPTB was used as screening mechanism. The fact that chest x-ray was not used in our study might underestimate the prevalence and incidence of TB in the area. The missing diagnosed TB cases at the baseline survey but which were later included in the prospective study might have resulted in an over-estimation of the TB incidence rate. Second, we excluded 20 contaminated sputum cultures from the analysis at the baseline survey and this may also have led to an underestimation of the prevalence of sputum culture TB. Third, we carried out a survey three times, first at the beginning of the study to determine the prevalence of TB, followed by the second at the end of the sixth month and the third at the end of the 12th month to estimate incidence of TB. However, the six-month time interval between surveys may have given sufficient time for spontaneous self-cure of active TB cases, which might have led to underestimation of the true incidence of TB cases in the study area. Fourth, we excluded 1,634 individuals who did not participate in the baseline survey from the subsequent follow-up study.

Moreover, 47 individuals who refused to participate in the follow-up study plus 18 deaths and 119 out-migrants were excluded from the analysis of the incidence rate due to the fact that the exact time of their contribution to the denominator was not known. Nonetheless, the overall proportion of participants excluded was only 5.5% and their baseline socio-demographic characteristics were similar to those included in the analysis. Therefore, their exclusion may not affect the overall findings of the study. Fifth, although HIV is a known risk factor for TB, we did not screen presumptive TB cases for HIV to measure the impact of HIV in fueling TB in the study area. Sixth, some relevant variables that might have affected the outcomes of interest were not included in the study. Hence, stratifying and analyzing only those included variables is less likely to fully control for other possible confounding variables and may introduce bias.

Conclusions and recommendations

The prevalence and incidence of smear-positive and bacteriologically confirmed TB cases were high in the study area. For every case of smear-positive TB receiving treatment, there was an almost equal number (0.96) of undetected infectious bacteriologically confirmed TB cases in the community. The high proportion of undetected infectious TB cases in the community could have resulted from the sub-optimal DOTS performance in detecting 70% of

infectious TB cases and attaining a cure rate of 85% in the study area. For this reason, there is a need to design an alternative strategy to improve TB case findings.A family history of contact has substantaially increased the risk of developing the disease, so there is a need to improve the identification of TB cases and intensify contact tracing among household members of PTB+ cases through the involvement of community-based health extension workers.

Declarations: The authors declare the ethics and consent to participate, the consent to publish, competing interests and the availability of data as follows.

Ethics and consent to participate

An official letter of ethical approval by the Institutional Review Board Committee at the Oromia Regional Health Bureau, Ethiopia, and subsequently by the Regional Committee for Medical and Health Research Ethics in Western Norway (REK Vest), and written consents obtained from participants were filed and kept at the Oromia Regional Health Bureau and can be presented for verification at any time.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The dataset supporting the conclusions of this article can be made freely available to any scientist wishing to use them for non-commercial purposes.

Authors' contributions

SDH was the principal investigator responsible for designing and conducting the study and was involved in analysing the data and writing the manuscript. BL participated in designing the study, analysing the data and writing the manuscript. MD participated in designing and

writing the manuscript. DT participated in the data collection and analysis. MS participated in the laboratory work. All authors read and approved the final manuscript.

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Paper IV

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RESEARCH ARTICLE Open Access

Primary and secondary anti-tuberculosis drug resistance in Hitossa District of Arsi Zone, Oromia Regional State, Central Ethiopia

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Abstract

Background: Multidrug-resistant tuberculosis (MDR-TB) drugs which is resistant to the major first-line anti-TB drugs, Isoniazid and Rifampicin, has become a major global challenge in tuberculosis (TB) control programme. However, its burden at community level is not well known. Thus, the aim of study was to assess the prevalence of primary and secondary resistance to any first line anti-TB drugs and MDR TB in Hitossa District of Oromia Regional State, Central Ethiopia.

Methods: Population based cross- sectional study was conducted on individuals aged ≥15 years. Those with symptoms suggestive of TB were interviewed and two sputum specimens were collected from each and examined using Lowenstein-Jensen (LJ) culture medium. Further, the isolates were confirmed by the Ziehl-Neelsen microscopic examination method. Drug susceptibility test (DST) was also conducted on LJ medium using a simplified indirect proportion method. The resistance strains were then determined by percentage of colonies that grew on the critical concentration of Isoniazid, Streptomycin, Rifampicin and Ethambutol.

Results: The overall resistance of all forms of TB to any first-line anti-TB drug was 21.7 %. Of the total new and previously treated culture positive TB cases, 15.3 and 48.8 % respectively were found to be a resistant to any of the first-line anti-TB drugs. Further, of all forms of TB, the overall resistance of MDR-TB was 4.7 %. However, of the total new TB cases, 2.4 % had primary while 14.3 % had secondary MDR-TB. Resistance to any of the first-line anti-TB drugs (adjusted odd ratio (AOR), 8.1; 95 % CI: 2.26–29.30) and MDR-TB (AOR), 7.1; 95 % CI: 2.6–43.8) was found to be linked with previous history of anti-TB treatment.

Conclusions: The study has identified a high rate of primary and secondary resistance to any of the first-line anti-TB drugs and MDR-TB in the study area. The resistance may have resulted from sub-optimal performance of directly observed treatment short-course (DOTS) programme in the detecting infectious TB cases and cure rates in the study area. Anti-TB drug resistance is linked with previous TB treatment. There is a need to strengthen DOTS and DOTS-Plus programmes and expand MDR-TB diagnostic facilities in order to timely diagnose MDR-TB cases and provide appropriate treatment to prevent the spread of MDR-TB in Ethiopia.

Keywords: Primary and secondary MDR-TB, Hitossa District, Ethiopia

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Background

Tuberculosis (TB) remains a high-priority communicable disease that causes illness among millions of people and is the second leading cause of death from an infectious disease worldwide [1]. The disease disproportionately affects people in resource-poor settings, particularly those in Asia and Africa. More than 80 % of TB cases and 78 % of deaths from the disease occurred in developing countries [2, 3]. In 2013, there were 9.0 million TB new cases, and 1.5 million TB deaths occurred around the globe [2]. Ethiopia has been listed as one of the 22 high-TB burden countries with respective mortality, prevalence and incidence rate of 32, 211 and 224 cases per 100,000 population. Moreover, 11 % of patients with TB are also infected by the Human Immunodeficiency Virus (HIV) [2].

Multidrug-resistant tuberculosis (MDR-TB) is defined as Mycobacterium tuberculosis strain resistant to at least the first-line anti-TB drugs of Isoniazid and Rifampicin. [1]. MDR-TB occurs either when a person is infected with a resistant strain or when insufficient or improper treatment leads to drug selection of the resistant strain [1]. When a person with no history of first-line anti-TB treatment develops MDR-TB, it is known as primary resistance to any first line anti- TB drugs and MDR-TB, whereas when a person with a history of first-line anti-TB treatment acquires resistance to any first line anti-TB drugs and MDR-TB, they are respectively called secondary resistance to any first line anti-TB drugs and MDR-TB [4].

Multidrug-resistant TB has been known to be a major challenge in TB control programme. It has been spreading rapidly across the globe, and in recent years an estimated 3.5 % of new cases and 20.5 % of previously treated TB cases have MDR-TB. In 2013, there were an estimated 480,000 MDR-TB cases, and about 210,000 deaths were caused by MDR-TB worldwide [2]. The prevalence of one or more drug-resistant TB and MDR-TB varies across counties with more than half of MDR-TB cases occurring in India, China and the Russian Federation [2]. The most difficult and complicated form of drug resistant TB is known as extensively drug resistant tuberculosis (XDR-TB); this has been reported from 92 countries (eight from Africa including Ethiopia) [5]. Globally 9 % of MDR-TB cases have XDR-TB [2, 6].

According to the 2014 World Health Organization (WHO) report, Ethiopia ranked 15th out of the 27 countries with the highest estimated number of multidrug resistant *tuberculosis* (MDR-TB) cases. Additionally, WHO estimated the prevalence of MDR-TB among new TB cases in Ethiopia at 1.6 % (95 % CI, 0.9 to 2.8 %), and among the previously treated ones at 12 % (95 % CI: 5.6 to 21 %) [2]. Furthermore, according to the 2014 National Anti-tuberculosis Drug Resistance Survey, the prevalence of MDR-TB among new, previously treated and overall TB cases were 2.3, 17.8 and 4.8 %, respectively [7]. Moreover, study reports from Eastern, Central, Northern and Southern Ethiopia have shown that the prevalence of primary resistance MDR-TB ranged from 1.1 to 5.8 % [8–11] and secondary resistance MDR-TB from 10.9 to 71.4 % [9, 10, 12, 13].

Nevertheless, to our knowledge, reports from Ethiopia on the prevalence of MDR-TB including primary and secondary resistance were based on reports among health service seekers from health facilities and might therefore be subject to selection bias [14–16].

Because of the absence of population-based studies and adequate laboratory testing facilities in the country, it is difficult to have a reliable estimation on the burden of primary and secondary resistance to any first line anti-TB drugs and MDR TB. However, this population-based study conducted in Hitossa District of Arsi zone Oromia Regional State, Central Ethiopia was the first attempt in the country aimed at measuring the prevalence of primary and secondary resistance to any first line anti-TB drugs and MDR-TB.

Methods

Study setting and population

The study was conducted in Hitossa District of Arsi Zone, Oromia Regional State in Central Ethiopia. The population density, socio–economic state, and demographic condition of Hitossa are typical representative of the entire features of Arsi Zone. The Directly Observed Treatment, Short-Course (DOTS) and health service coverage, the proportion of urban–rural population and HIV prevalence, and the TB case notification and treatment outcomes of Hitossa District are also similar to those of the whole zone of Arsi. The district has an estimated population of 178,229 people living in 23 rural and one urban *kebele* (the smallest administrative unit in government structure) [17]. Since 2010, *kebeles* have been further divided into three sub-*kebeles* known as *gote*. Each *gote* has about 1/3 population of the *kebele* [17].

Study design

Population based cross sectional study was conducted between 1 July 2013 and 30 June 2014 to estimate the prevalence of primary and secondary multi-drug resistant to any first line anti-TB drugs and MDR-TB in Hitossa District of Arsi Zone. For practical and economic reasons Hitossa District was selected purposefully and all its *kebeles* were included in the study. One *gote* from each *kebele* was randomly selected, and individuals 15 years and above living in the households of the selected *gote* were enrolled in the study. A week before the baseline survey, pre-survey registration was carried out in the selected *gote*s of the 24 *kebeles*. Accordingly, 61,678 individuals living in 9,454 households were

identified. Eligibility includes permanent residents of 15 years of age and above who could provide written consent for willingness to participate in the study and temporary visitors who arrived at least 15 days before the commencement of the study. Using the above criteria, 33,073 adults were identified and included in the study.

Data collection procedures

The aims of the study and the procedures of data collection were discussed with zonal, district and *kebele* leaders. A total of 24 teams with one nurse and one health extension worker (HEW) were involved in the data collection. Furthermore, five laboratory technicians also took part in sputum sample collection. The whole process of data collection was supervised by 10 health officers. All laboratory technicians and data collectors were selected from public health institutions in the study district, and they were trained on TB screening techniques and on how to collect and transport sputum specimen. The same team of data collectors was assigned to each *kebele* for both pre-survey registration and data collection. The survey was carried out three times at interval of 6 months: the first at baseline, the second at the end of the sixth month, and the third at the end of the 12th month between 1 July 2013 and 30 June 2014.

House to house visits were carried out to identify individuals with persistent cough of more than two weeks, fever, and loss of appetite, weight loss, blood-stained sputum and chest pain or difficulty of breathing which were considered as symptoms suggestive of pulmonary TB. Individuals with such symptoms were interviewed on their socio–economic and demographic information and current and previous history of TB treatment. Subsequently, participants with any symptom suggestive of the illness were asked to submit two adequate recently-discharged mucoid or muco-purulent sputum specimens (spot-morning). The laboratory technicians collected the sputum in sterile falcon tubes and immediately placed them in a cold box at 4 °C, and transported them on the same day to Adama Regional Research Center Laboratory.

Culture and identification

On the following day of the sputum receipt, the morning specimen was digested and decontaminated by the standard Acetyl L-cysteine (NALC)-NaOH method [18], followed by centrifuged at 800 X g for 15 min to concentrate the organisms. The sediment (pellet) was reconstituted with 2.5 ml of sterile phosphate buffer (pH 6.8) to prepare the suspensions for the cultures. The sediments were inoculated in to egg-based Löwenstein-Jensen (LJ) medium slant tubes prepared based on the International Union against Tuberculosis and Gung Disease (IUATLD) for the primary isolation of the organisms [19]. Following that the LJ slant tubes were incubated at 37 °C and inspected for a period of eight weeks for the growth of *Mycobacterium tuberculosis* complex. The cultures were considered to be negative if no colonies were identified after 8 weeks of incubation. Moreover, the isolates from the LJ were confirmed by microscopic examination for the presence of Acid-Fast Bacillus (AFB) using Ziehl-Neelsen method. *Moreover*, susceptibility test of all isolates to spara-nitrobenzoic acid was carried out to identify *Mycobacterium tuberculosis complex* from environmental mycobacteria. However, all isolates were found to be *Mycobacterium tuberculosis complex* [19].

Drug susceptibility test

Drug susceptibility tests(DST) were carried out using the simplified indirect proportion method on LJ medium [19]. The proportion method validates the percentage of growth of distinct inoculums on a drug-free control medium compared to growth on culture media containing the critical concentration of anti-tuberculosis drugs. The resistant strains were determined using the percentage of colonies that grew on the critical concentration of 0.2 mg/l for Isoniazid, (INH), and 4 mg/l for Streptomycin (STM), 40 mg/l for Rifampicin (RIF) and 2 mg/l for Ethambutol (EMB). The isolate was said to be drug resistant when the growth was more than or equal to 1 % of the bacterial population on the media containing the critical concentration of each drug [19].

Data management and analysis

To ensure data quality, the principal investigator and supervisors closely monitored the data collection process in which a standardized and pre-tested questionnaire was used. The collected data were coded and double entered into Epi-info version 7 statistical software by trained data clerks. Then they were checked against the original information for missing variables. Errors were corrected by referring to the original questionnaire.

The percentage of drug resistance to any of the drugs or its combination with other drugs was determined. The primary resistance was calculated by dividing the number of resistant isolates among new TB cases by the total number of new TB cases found to be culture positive for a particular drug or combination of drugs multiplied by 100. Similarly, percentage of secondary resistance was calculated by dividing the number of resistant isolates in previously treated TB cases by the total number of re-treated TB cases found to be culture positive for that particular drug or combination of drugs multiplied by 100. We excluded 20 contaminated cultures from the analysis as their results were not known.

Analysis was made using IBM SPSS version 20 statistical software (SPSS Inc. Chicago. 2007). Descriptive

analysis was made and frequencies and odds ratios (OR) with the 95 % confidence intervals (CI) were calculated. Logistic regression analysis was used to evaluate the association between drug resistance as outcome and others related independent variables. A significance level of <0.05 was considered statistically significant. We also compare the result of current study with those of other studies carried out in Ethiopia between 1984 and 2015 to understand the trend of drug resistance over time (Table 4).

Results

General characteristics of the study population

A total of 61,678 individuals in 9,454 households from the study area were identified. Of these, 33,073 were found to be eligible, and screened for symptoms suggestive of PTB. Of these, 16,888 (51 %) were males and 28,048 (84.8 %) were rural residents. The age of the respondents ranged from 15 to 94 years, with a mean age of 42.3 (±18.4 SD) years. Out of the eligible individuals, 2,758 (8.3 %) reported to have symptoms suggestive of PTB. Among these, 1,717 (62.3 %) were females and 1041 (37.7 %) were males. Of the 2,758 suspected cases, 2, 218 (80.4 %) were new, and 540 (19.6 %) had previously been treated with first-line anti-TB drugs (Table 1).

Table 1 General characteristics of TB suspects, smear culture positive and MDR-TB cases in Hitossa District, Arsi Zone of Oromia Region, Central Ethiopia, 2015

Characteristics	TB suspects ($N = 2,758$)	Culture positive TB cases ($N = 106$)
Sex	n (%)	n (%)
Male	1041 (37.7)	51 (48.1)
Female	1717 (62.3)	55 (51.9)
Total	2758 (100)	106 (100)
Age		
15–24	543 (19.7)	40 (37.7)
25–34	525 (19.0)	18 (17.0)
35–44	448 (16.3)	29 (27.4)
≥ 45	1242 (45.0)	19 (17.9)
Total	2758 (100)	106 (100)
Residence		
Rural	2277 (82.6)	58 (54.7)
Urban	481 (17.4)	48 (45.3)
Total	2758 (100)	106 (100)
Education		
Literate	1212 (43.9)	47 (44.3)
Illiterate	1546 (56.1)	59 (55.7)
Total	2758 (100)	106 (100)
History of pervious TB treatment		
No	2218 (80.4)	85 (80.2)
Yes	540 (19.6)	21 (19.8)
Total	2758 (100)	106 (100)

Drug susceptibility pattern

All the 2,758 TB suspect cases gave spot and morning sputum for culture examination. Of these, 106 (3.8 %) were found to be culture positive for *Mycobacterium tuberculosis*. Twenty specimens were contaminated and were therefore excluded from the analysis. From the total 106 culture positives, 85 (80.2 %) were new, whereas 21 (19.8 %) were previously treated patients with first-line anti-TB drugs (Table 1). Of the 106 isolates, 83 (78.3 %) were susceptible to all first-line anti-TB drugs (Streptomycin (STM), Isoniazid (INH), Ethambutol (EMB) and Rifampicin (RIF)), while 23 (21.7 %) were resistant to one or to a combination of the first-line anti-TB drugs. Five cultures (4.7 %, 95 % CI: 2.8–6.6 %) were MDR-TB cases.

Of the 85 new *M. tuberculosis* isolates, primary resistant strains to any first-line anti-TB drugs were observed in 13 (15.3 %) patients. Of these, 8 (9.4 %) were resistant to each INH and STM, 4 (4.7 %) to RIF and 3 (3.5 %) to EMB. Primary MDR-TB was detected in 2 (2.4 %) strains (Table 3). However, among the new cases, no isolates of primary resistance to all first-line drugs were observed. Primary mono-resistance to each of INH and STM was found in two cultures (2.4 %). There was no primary mono-resistance to RIF and EMB among the new isolates (Table 2).

Moreover, out of the 21 previously treated *M. tuberculosis* isolates, strains of secondary resistance to any of the first-line anti-TB drugs were identified in 11 (52.2 %) patients. Of these, 6 (28.6 %), were resistant to INH, 5 (23.8 %) to STM, and 4 (19 %) to RIF. Secondary MDR-TB was detected in 3 (14.3 %) isolates. Of these, one (4.8 %) was found to be resistant to all first-line drugs (Table 3). Among previously treated cases, the highest mono-resistance was to STM with 2 (9.5 %), followed by one (4.8 %) to each INH and EMB. However, among the previously treated TB cases, no mono-resistance to RIF was reported (Table 2).

Risk factors associated with drug resistance

Previous history of TB treatment, and urban residence were independently associated with high risk of resistance to any first-line anti-TB drug. Individuals with pervious history of TB treatment were eight times (adjusted odd ratio (AOR), 8.1; 95 % CI: 2.3–29.3) more likely to develop resistance to any first-line anti-TB drugs compared to those with no history of previous TB treatment. Similarly, urban residents were four times (AOR, 4.1; 95

Table 2 Primary and secondary drug resistance pattern to first-line anti-TB drugs among culture positive pulmonary TB cases in Hitossa District, Arsi Zone of Oromia Region, Central Ethiopia 2015

Drug resistance pattern	New cases (*N* = 85)	Re-treated cases (N-21)	Total (*N* = 106)
	n (%) (95 % CI)	n (%) (95 % CI)	n (%) 95 % CI
Any R to one drug	13 (15.2 % (7.6–22.8)	11 (52.3: 30.9–73.7)	24 (22.6: 14.6–30.6)
Any INH	8 (9.4 %)	6 (28.6)	14 (13.2)
Any RIF	4 (4.7)	4 (19.0)	8 (7.5)
Any STM	8 (9.4)	5 (23.8)	13 (12.3)
Any EMB	3 (3.5)	2 (9.5)	5 (4.7)
Mono resistance	4	4	8
Only INH	2 (2.4)	1 (4.8)	3 (2.8)
Only RIF	0	0	0
Only STM	2 (2.4)	2 (9.5)	4 (3.8)
Only EMB	0	1 (4.8)	1
Two-drug resistance	7	4	11
INH + RIF	1 (1.2)*	1 (4.8)	2 (1.9)*
INH + ETM	1 (1.2)	0	1 (0.9)
INH + STM	2 (2.4)	2 (9.5)	4 (3.8)
RIF + EMB	0	0	0
RIF + STM	2 (2.4)	1 (4.8)	3 (2.8)
ETM + STM	1 (1.2)	0	1 (0.9)
Three or more-drug resistance	2	3	5
INH + RIF + EMB	0	0	0
INH + RIF + STM	1 (1.2)*	2 (9.5)	3 (2.8)*
INH + EMB + STM	1 (1.2)	0	1 (0.9)
RIF + ETM + STM	0	0	0
INH + RIF + EMB + STM	0	1 (5.9)	1 (0.9)
MDR*	2 (2.4)	3 (14.3)	5 (4.7)

NB: MDR-TB* is multi-drug resistant TB

% CI: 1.3–12.8) more likely to have resistance to any first-line anti-TB drugs compared to their rural counterparts. Individuals who had pervious history of TB treatment were nearly seven times more likely (AOR 7.1; 95 % CI: 2.6–43.8)) to have MDR-TB compared to those who had no history of pervious exposure to anti-TB drugs (Table 3).

Rates of anti-TB drug resistance between 1984 and 2015

A total of 18 studies that had been published between 1994 and 2015 on the primary and secondary anti-TB drug resistance were reviewed. Results showed that primary resistance to any drug raged from 10.7 % in 2009 to 30.1 % in 2012. Moreover, the rate of primary MDR-TB increased from 0.6 in 1994 to 3.7 % in 2009 while that of secondary resistance to any anti-TB drugs varied from 11.1 % in 1996/7 to 85.7 % in 2005/6. According to reports from the same studies, the rate of secondary MDR-TB ranged from 15.7 % in 1996/7 and 60.8 % in 2005/6 (Table 4).

Discussions

Fifteen percent of the newly diagnosed, and fifty two percent of the previously treated TB cases were resistant to one or more of the first-line anti-TB drugs. MDR TB among new cases was 2.4 %, and 14.3 % among previously treated patients. Drug resistance was associated with previous history of TB treatment and urban residence. Resistances to any one or more of first-line anti-TB drugs and MDR-TB in the study population were high.

In this study, the 15 % resistance to any of the first-line anti-TB drugs among new TB cases is comparable to reports from Addis Ababa and Northern Ethiopia [10, 20]. However, it is higher than reports from other African countries [21–23] and yet lower than those from different parts of the country and elsewhere in Africa [8, 10, 11, 13, 24–29]. Moreover, the 52 % resistance rate to one or more first-line anti-TB drugs among previously treated TB cases in our study is high compared to previous reports from Ethiopia and others African counties [28–30]. However, it

Table 3 Pattern of drug resistance among culture-positive TB cases with different variables in Hitossa District, Arsi Zone of Oromia Region, Central Ethiopia, 2015

Characteristics	Culture positive ($n = 106$)	MDR-TB cases ($n = 5$)			Any TB drug resistance ($n = 23$)		
		Number	COR (95 % CI)	AOR (95 % CI)	Number	COR (95 % CI)	AOR (95 % CI)
Sex							
Male	51 (48.1)	2	0.71 (0.11–4.4)	0.67 (0.10–4.2)	14	1.54 (0.61–3.90)	2.5 (0.78–8.05)
Female	55 (51.9)	3	1.00	1.00	10	1.00	1:00
Total	106 (100)	5			24		
Age							
15–24	40 (37.7)	3	1:00	1.00	10	1.00	1:00
25–34	18 (17.0)	0	———————	———————	6	1.5 (0.45–5.10)	2:18 (0.55–8.70)
35–44	29 (27.4)	2	0.91 (0.14–5.84)	0.90 (0.3–6.1)	6	0.63 (0.19–2.10)	0.36 (0.08–1.56)
≥ 45	19 (17.9)	0	———————	———————	2	0.35 (0.07–1.80)	0.19 (0.03–1.35)
Total	106 (100)	5			24		
Residence							
Rural	58 (54.7)	1	1:00	1.00	9	1.00	1:00
Urban	48 (45.3)	4	5.18 (0.56–4.8)	4.8 (0.7–4.7)	15	2.84 (1.11–7.45)	4.1 (1.33–12.84)
Total	106 (100)	5			24		
Education							
Literate	47 (44.3)	3	1:00	1.00	10	1.00	1:00
Illiterate	59 (55.7)	2	0.51 (0.08–3.21)	0.63 (0.09–3.6)	14	1.31 (0.51–3.37)	1.64 (0.53–5.11)
Total	106 (100)	5			24		
History of TB treatment							
No	85 (80.2)	2	1:00	1.00	14	1.00	1:00
Yes	21 (19.8)	3	6.92 (1.10–44.4)	7.1 (2.6–43.8)	10	3.48 (1.29–9.44)	8:13 (2.26–29.30)
Total	106 (100)	5			24		

COR Crude Odds Ratio and *AOR* Adjusted Odds Ratio

is lower than 54 % reported from Benin [31], 53.8 % from Somalia [32], 58.5 % from northern Ethiopia [10], the 71.4 and 72 % from Addis Ababa City [9, 13].

The difference in resistance rate to one or more first-line anti-TB drugs among new and previously treated TB patients across different study settings could be attributed to the variation in TB control programme performance, study population, sample size and study methods that were used across different geographical settings. For instance, the study subjects from Addis Ababa who had high rate of resistance stain [9, 13] were presumptive MDR-TB cases referred for MDR-TB investigation, while those recruited for the current study included any person 15 years age and above in the general population who had symptoms of TB and was at low risk of drug resistance.

Moreover, previous studies from Ethiopia and elsewhere in Africa were restricted to health service seekers at health facilities. Thus, study results from such segment of population may not indicate the real burden of the disease at community level as compared to the present population-based study. Likewise, the difference in burden of the resistance cases could be due to the time of the study; in the past the prevalence of resistant strains which might be a potential source of infection was not as high as they are today. For instance, in 2001, resistance to any first-line anti-TB drug among previously treated TB patients in Arsi Zone was 31.6 % [24]. However, after 14 years, it has reached 52.3 % in the study area. In general, the rate of primary and secondary resistance has been increasing in Ethiopia over the twenty years [7–12, 33–38]. Table 4 summarizes reports of previous studies on primary and secondary resistance compared to the current study in Ethiopia.

In the present study, previously treated TB cases were eight times more likely to have resistance to any of the first-line anti-TB drugs compared to new ones. The high level of anti-TB drug resistance among previously treated TB cases might have resulted from poor adherence and follow up or inadequate drug supply. This might result in selection of spontaneous mutation of *M. tuberculosis* strains [39]. Hence, the TB control programme has to explore reasons for such high secondary anti-TB resistances in the study area.

Table 4 Rate of anti-TB drug resistance in current and other studies conducted between 1984 and 2015 in Ethiopia

Type of the study		Study time	Sample size	Rate of anti-TB resistance					
				Primary resistance		Secondary resistance		Overall resistance (primary plus secondary resistance)	
Community-based	Institution-based			Any-resistance (%)	MDR-TB (%)	Any- resistance (%)	MDR-TB (%)	Any- resistance (%)	MDR-TB (%)
Current study		2014	33073[a]	15.3	2.4	48.8	14.3	21.7	4.7
	Demissie M et al. [34]	1994	167[b]	15.6	0.6				
	Bruchfeld et. al [22]	1996–1997	509	14.6	0.9	11.1	0	15.7	1.7
	Abate et al. [35]	1998	30[c]					50	12
	Demissie et al. [40]	1998		12.9	0.6				
	Gebeyehu M et al. [23]	2001	203	18.2	0	31.6	0	19.5	0
	First National Drug resistance survey [7]	2003–2005	804		1.6		11.5		
	Desta K et al. [36]	2004–2005	297[b]	27.4	0				
	Abate D et al. [13]	2004–2008	376[c]			72.9	46.3		
	Asmamaw et al. [25]	2004–2005	173[b]	21.4	0.6				
	Agonafir M et al. [9]	2005–2006	114[c]	25	2.3	85.7	63.5	60.8	38.3
	Tessema B et al. [10]	2009	260	10.7	3.7	39.1	10.9	15.8	5.0
	Abebe G et al. [11]	2010–2011	136[b]	18.4	1.5				
	Esmael A et al. [37]	2010–2011	230	23.6	1.8	58.5	18.5	33.5	6.5
	Yimer et al. [26]	2012	112[b]	30.1	1.0				
	Seyoum et al. [8]	2011–2013	408[b]	23	1.1				
	Nigus et al. [12]	2012–2013	606[b]				15.3		
	Second National Drug resistance survey (7)	2014	1651		2.3		17.8		
	Mulisa G et al. [38]	2015	439[c]				33.2 %		

[a] Of the study population, 106 TB cases were diagnosed
[b] Only new cases
[c] Previously treated and now presumptive for MDR-TB

Moreover, resistance to any of the first-line anti-TB drugs was higher among urban residents when compared to their rural counterparts. This is in agreement with findings from a previous study in eastern Ethiopia which reported resistance of 81.7 % among urban dwellers 18.3 % among rural residents [8]. In fact, the association between MDR-TB and urban residence is not well established in the literature and may need further investigation. However, the most likely reason for the high prevalence of MDR-TB among urban residents might be because of higher HIV prevalence in urban settings and this may have increased the risk of MDR-TB infection [27, 40]. Moreover,, the high probability of exposure to anti-TB drugs from different sources in urban setting and the crowded living condition may also have contributed to increased transmission of resistant strains.

The 2.4 % prevalence of MDR-TB among the newly diagnosed TB cases in this study is very high compared to the 0 % reported in 2001 from the same area [24]. Nevertheless, it is similar to the 2.3 % reported by the 2014 National Drug Resistance and also by a study conducted in Addis Ababa [7, 9]. The relatively high rate of primary MDR-TB cases in the current population-based study could be due to the identification of the undiagnosed resistance cases in the community. Thus, these findings may imply the need for intensifying active TB case finding using community-based health extension workers and this could help in timely identification of undiagnosed resistant strains in Ethiopia [33].

The 14 % prevalence of MDR-TB among previously treated TB cases obtained in this study is lower than 18 % reported by the national surveillance [7], the 20.5 % of the global estimate [1] and other reports from elsewhere [30, 32, 41, 42]. However, it is high compared to the 0 % prevalence report from Arsi zone in 2001 [24]. Secondary resistance is mainly a result of poor Directly Observed Treatment Short course (DOTS) programme and should be as taken as a serious challenge in the TB control programme.

In this study, previously treated TB cases were more likely to have MDR-TB than the new ones. This is

consistent with a meta-analysis in sub-Saharan Africa and a systematic review in Europe where a pooled risk of MDR-TB was higher among the previously treated TB cases as compared to the new ones [43, 44]. The overall high level of anti-TB drug resistance among re-treated TB cases compared to the newly diagnosed ones might be an indication of sub-optimal DOTS and DOTS-plus programme performance in Ethiopia.

The DOTS strategy aims to detect 70 % of infectious TB cases and achieve 85 % cure rate in order to interrupt transmission, reduce mortality and avert the emergence of drug resistance [45, 46]. However, reports from previous studies conducted in Ethiopia between 1984 and 2015 showed that the proportion of MDR-TB among new and previously treated TB cases varies from place to place and increased over time (Table 4). For instance, over 13 years, it increased from 0 to 2.4 % among new patients and from 0 to 14.3 % among previously treated ones in Arsi Zone [24]. Furthermore, according to previous reports, the fifteen years average TB case notification and cure rate of the study area were as low as 51.8 and 66.9 % [47, 48] respectively, very far from the 70 % global target of TB case notification and 85 % cure rate. Thus, the increasing trend in resistance to any TB drug and MDR-TB over time with the low TB case notification and cure rate in the study area may warrant alternative strategy to avert the emergence of drug resistance and strengthen TB control programme in Ethiopia.

Although DOTS- plus strategy is believed to be the best strategy to prevent emergence of MDR TB by accessing presumptive MDR-TB cases to diagnostic and appropriate treatment, MDR-TB patients are challenged by much more toxic and complicated treatment of longer duration which results in poor treatment outcome and emergence of XDR-TB. Therefore, effective implementation of DOTS and DOTS-plus strategies which are believed to be a corner stone in the prevention of the emergence and spread of MDR-TB and XDR-TB should be strengthen in the country. Thus, expanding MDR-TB diagnostic facilities and intensifying active case findings using health extension workers is urgent issues to be addressed in order to effectively control the increasing trend of drug resistance TB in Ethiopia [33].

To our knowledge, this is the first population-based study that analysed the prevalence of primary and secondary drug resistance and MDR-TB in Ethiopia. It might also be one of the very few studies conducted in poor resource setting. In fact, experienced and qualified laboratory technicians carried out the smear microscopy, sputum culture and DST to isolate *M. tuberculosis* and drug resistant strain. Thus we believe that the finding has highlighted the real burden of anti-TB drug resistance at community level. However the study is not without limitation. First, symptoms suggestive of TB were used as screening mechanism. The fact that chest X-ray was not used in the current study might have resulted in missed asymptomatic TB cases and underestimate the burden of the disease. Second, the current study excluded 20 contaminated sputum cultures which may have an impact on our result.

Conclusions

The study has identified a high rate of primary and secondary resistance to any of the first-line anti-TB drugs and MDR-TB in the study area. The high rate of anti-TB drug resistance may have resulted from sub-optimal performance of DOTS programme in detecting infectious TB cases, and low cure rate in the study area. As anti-TB drug resistance is linked with pervious TB treatment, there is a need to strengthen DOTS and DOTS-Plus programme and expand MDR-TB diagnostic facilities so as to detect the cases in time and start appropriate treatment to prevent the spread of MDR-TB in Ethiopia.

Additional files

Additional file 1: Dataset supporting conclusions of the study on primary and secondary MDR-TB of Hitossa District of Arsi Zone, 2016, submitted to BMC public health using STATA software. (DTA 16 kb)

Additional file 2: Dataset supporting conclusions of the study on primary and secondary MDR-TB of Hitossa District of Arsi Zone, 2016, submitted to BMC public health using excel software. (CSV 22 kb)

Abbreviations

AFB, Acid-Fast Bacillus; AOR, adjuster odd ratio; CI, confidence intervals; DOTS, directly observed short course treatment; DST, drug susceptibility tests; EMB, ethambutol; HEW, health extension worker; HIV, Human Immunodeficiency Virus; INH, isoniazid; IUATLD, International Union against Tuberculosis and Gung Disease; LJ, Löwenstein-Jensen; MDR-TB, multi- drug-resistant TB; RIF, rifampicin; STM, streptomycin; TB, tuberculosis; WHO, World Health Organization; XDR-TB, extensively drug-resistant TB

Acknowledgements

We would like to thank the Oromia Regional Health Bureau and the staff of respective health facilities involved in the study for their support during the field data collection. We also like to extend our gratitude for the Management science for Health (MSH) Heal TB project for their financial support to this study.

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Availability of data and material

The dataset supporting the conclusions of this article is included within the article as an additional supporting file. The data is under Additional files 1 and 2.

Authors' contributions

SDH was the principal investigator responsible for designing and conducting the study. Further, he was involved in analysing the data and writing the manuscript. BL participated in designing the study, analysing the data and writing the manuscript; MD participated in designing and writing the manuscript. DT

participated in data collection and analysing the data and MS participated in laboratory work. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
The study protocol was first reviewed and approved by the Institutional Review Board Committee at Oromia Regional Health Bureau, Ethiopia, and subsequently by the Regional Committee for Medical and Health Research Ethics in Western Norway (REK Vest). Written consent from participants aged ≥18 years and assent and consent from the guardians or caretakers of participants aged less than 18 years were obtained before data collection. Data on individual information were analysed and reported anonymously. Immediate referrals were arranged for participants found to be culture positive and all TB cases started anti-TB treatment at health centres close to them.

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Questionnaire for 15 years TB case notification and treatment outcomes.

(Paper I and II)

Ser. No.	Questions	Variables
1	Patient Code	_____
2	Woreda/Town	_____
3	Health Center/Hospital	_____
4	Address of the patient	1. Urban 2. Rural
5	Sex	1. Male 2. Female
6	Age	_____ yrs
7	Did the patient have a contact person	1. Yes 2. No
8	Smear result	1. PTB+ 2. PTB- 3. Smear not done/not registered
9	Weight	_____ kgs
10	Category /Case definition	1. New (N) 4. Defaulter (D) 2. Relapse(R) 5. Transfer in (T) 3. Failure(F) 6. Other cases(O)
11	Type of TB	1. P/Pos 3. EPTB 2. P/Neg 4. Not registered
12	Intensive phase drug type	1. RHZE 3. S (RHZE) 4. S (RHZ) 2. RHZ 5. Other regimen, specify
13	Treatment started date	DD_____MM_____ YY_____
14	Dose(no. of days) of intensive phase treatment	_____ Days
15	Did the patient interrupt treatment during intensive phase	1. Yes 2. No
16	If yes for question No 14, for how long?	_____days
17	Was the patient tested for HIV	1. Yes 2. No
18	If yes Q. no. 16 what was the result?	1. HIV positive 2. HIV negative
19	Follow up sputum examination at end of 2nd /3rd month	1. Negative 3. Not done/ not registered 2. Positive
20	Follow up sputum examination at end of 5th month	1. Negative 3. Not done/ not registered 2. Positive
21	Follow up sputum examination at end of 7nd month	1. Negative 3. Not done/ not registered 2. Positive
22	Follow up weight at end of 2nd month	1. _____ kgs 2. Not measured/recorded
23	Follow up weight at end of 5th month	1. _____ kgs 2. Not measured/recorded
24	Follow up weight at end of 7nd month	1. _____ kgs 2. Not measured/recorded
25	Continuation phase drug	1. EH 3. RH, H 2. RH 4. Other regimen specify_____
26	How long the patient did received drug during continuation phase?	_____ months
27	Treatment Outcome	1. Cured 4. Failure 2. Rx completed 5. Transfer out 3. Died 6. not registered/evaluated

HEALTH CENTER/HOSPITAL UNIT TUBERCULOSIS REGISTER

Federal Ministry of Health

MRN	Unit TB No.	Name of the patient	Sex (M/F)	Name of contact person	Smear result	Category N.R.F.D.T.O	Intensive phase		Treatment started (DD/MM/YY)	Write the month	Intensive phase treatment monitoring chart																													
		Address of the patient (Woreda, Kebele, HNo)	Age	Address contact person (Woreda, Kebele, HNo)	Lab. no.	P/Pos, P/Neg or EP	Drug	Dose			Days:																													
					Weight						1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(22)	(23)	(24)	(25)	(26)	(27)	(28)	(29)	(30)	(31)	(32)	(33)	(34)	(35)	(36)	(37)	(38)	(39)	(40)	41

HEALTH CENTER/HOSPITAL UNIT TUBERCULOSIS REGISTER

Federal Ministry of Health

HIV test offerred (√)	HIV test performed (√)	HIV test result (R or NR or I)	CPT started (DD/MM/YY)	Enrolled in HIV care (DD/MM/YY)	ART started (DD/MM/YY)	Sputum results, lab. name, serial nr.& wt			Continuation phase		Continuation phase treatment monitoring chart 4 - weekly attendance (for category II three times per week)													Write the date (DD/MM/YY) that treatment was stopped in appropriate column:						Remarks
						Months			Drug	Dose	Month:													Cured	Treatment Completed	Died	Failure	Defaulted	Transferred out	
						2nd	5th	7th			Ham	Neh	Pag	Mes	Tik	Hid	Tah	Tir	Yek	Meg	Mia	Gin	Sen							
(42)	(43)	(44)	(45)	(46)	(47)	(48)	(49)	(50)	(51)	(52)	(53)	(54)	(55)	(56)	(57)	(58)	(59)	(60)	(61)	(62)	(63)	(64)	(65)	(66)	(67)	(68)	(69)	(70)	(71)	(*72)

Questionnaire for TB prevalence, Incidence (paper III) and primary and secondary multi resistance TB (MDR-TB (paper IV))

Id number	
Date of interview	
Cluster site number	
Serial number / house number	
Name of data collector	
Data collection round	Round 1, Round 2 , Round 3

1. Demographic data

1.1	Age of the patient	----------years old
1.2	Sex	1. Male ---------- 2. Female ----------
1.3	Ethnicity	1. Oromo 2. Tigre 2. Amahara 3. Others
1.4	Marital status	1. Single 3. Divorced 2. Married 4 .Widowed
1.5	Residence	1. Urban 2. Rural
1.6	Educational status	1. illiterate 2. Read and write 3. Primary 4.Secondary 5. post-secondary
1.7	Occupation	1. peasant 2. Student 3. Petty tread 4.civil servant 5.private business 6. unemployed

2 TB related symptoms

2.1	Do you have cough?	1. Yes ---- --- 2. No ----- ----- , if no go to section 3
2.2	Do you have for more than 3 weeks?	1. Yes -------- 2. No----------
2.3	Do you have productive cough (sputum)?	1. Yes ------- 2. No---------
2.4	Do you have blood stained sputum?	1. yes --------- 2.No--------
2.5	Do you have chest pain in the last 4 weeks?	1. yes --------- 2.No--------
2.6	Do you have fever for more than 2 weeks?	1. yes --------- 2.No--------
2.7	Do you have night sweating for more than 2 weeks?	1. yes --------- 2.No--------
2.8	Do you have weight loss more than 3kg in the past 4 weeks?	1. yes --------- 2.No--------

2.9	Do you have loss appetite in the past 4 weeks?	1. yes --------- 2.No--------
If the patient has suggestive of TB symptoms as him or her to submit sputum for bacteriological examination (smear microscopy/culture		

3. Previous history of TB contact

3.1	Is there any household member currently taking TB treatment ?	1. yes --- 2.no ------ 3. If yes, who is on anti-TB treatment ---
3.2	Is there any household member previously treated for TB ?	1. yes------ 2. No--- 3. if yes, who ----- 4 if yes when ------
3.3	Has any adult household member has persistent cough for more than 2 weeks in the last month?	1. yes ------ 2.No------
3.4	Have you ever been hospitalized for other than TB?	1. yes --------- 2. No------

4. Previous History of TB Treatment

4.1	Is the patient currently diagnosed with TB and on anti-TB treatment?	1. yes------ 2. No ---------
4.2	If yes, type of TB?	1. pulmonary smear positive 2. pulmonary smear negative 3. extra-pulmonary
4.3	If yes what is the treatment	1. category I 2. category II 3. other , specify
4.4	Is the patient previously diagnosed with TB?	1. Yes ------ 2. No-------
4.5	If yes, type of TB?	1. pulmonary smear positive 2. pulmonary smear negative 3. extra pulmonary
4.6	If yes what was the treatment?	1. category I 2. category II 3. other , we don't know
4.7	Date of stopping pervious treatment	
4.8	What was then pervious TB treatment outcomes?	1. treatment completed 2. treatment cured 3. treatment failure 4. Treatment defaulted 5. Other , unknown

1. Date----------

2. Signature ----------

3. Name of the investigator----------

Appendices II: Ethical approvals

BIIROO EEGUMSA FAYYAA OROMIYAA

OROMIA HEALTH BUREAU
የኦሮሚያ ጤና ጥበቃ ቢሮ

Lakk/Ref. No. BEFO/HBTFH/1-8/2301
Guyyaa /Date 26/01/2013

Waaj/Eeg/Fay/God/ Arsii tiif

Assallaa

Akkuma beekamu Biiroon keenya ogeeyyii, dhaabbilee akkasumas namoota qorannoo gaggeessuuf piroppoozaala dhiyeeffatan piroppoozaala isaanii madaaluun akkanumas iddoo biraatti ilaalchisanii fudhatama argatee (approved) dhiyaateef, piroppoozaala isaanii ilaaludhaan waraqaa deeggarsaa ni-kenna. Haaluma kanaan mata-duree**''Improved Tuberculosis control programme in Ethiopia, analyzing Tuberculosis programme performance and estimating the prevalence of pulmonary tuberculosis and MDR-TB in Arsi zone, Oromia Regional State'' Obbo Shaalloo Dhaabaa'' godina keessan** keessatti qorannoo geggeessuuf piroppoozaalii isaanii Koree "Health Research Ethical Review Committee" Biiroo keenyaatti dhiyeeffataniiru. Haaluma kanaan Koreen "Health Research Ethical Review Committee" Biiroo keenyaas piroppoozaala kana ilaaluun mirkaneesse qorannoon kun akka hojiirra oolu murteessee jira. Waan kana ta'eef hojii qorannoo kanarratti deggerssa barbaachisaa ta'e akka gootaniif jechaa'' **Obbo Shaalloo Dhaabaa'''s** wayitii qorannoon kun qaacceffamee xumurame fiiriisaa Biiroo Eegumsa Fayyaa Oromiyaa fi iddoo qorannoon irratti adeemsifameef kooppii tokko tokko akka galii goodhan garagalchaa xalayaa kanaatiin isaan beeksifna.'' **Obbo Shaalloo Dhaabaa'''s** wayitii qorannoon kun qaacceffamee xumurame fiiriisaa kooppii tokko tokko Biiroo Eegumsa Fayyaa Oromiyaa fi iddoo qorannoon irratti adeemsifameef akka galii godhu mallattoo kiyyaan mirkaneessa.

Nagaa wajjin

Gammachuu Shuumii
Eggeesaa Adeemsa Hojii Ijoo Balaa
Tasaa Fayyaa Hawaasaa Qu' annoo
fi Qorannoo Fayyaa (BSC,MPH)

Mallattoo_____
Maqaa'' **Obbo Shaalloo Dhaabaa''**
Guyyaa _____
Lakk. Bilbilaa_ **0911-52-38-18**

GG

'' Obbo Shaalloo Dhaabaa'' tiif
Bakka jiranutti

Tessoo: Tel: 011-371-72-77, Fax 011-371-72-27 Box. 24341 E-mail: **ohbhead@telecom.net.et**
Address: ADDIS ABABA/FINFINNE-ETHIOPIA

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK vest	Øyvind Straume	55978496	04.09.2014	2014/1048/REK vest
			Deres dato:	**Deres referanse:**
			17.06.2014	

Vår referanse må oppgis ved alle henvendelser

Bernt Lindtjørn
Postboks 7804

2014/1048 Forbedre tuberkulosekontroll i Arsi regionen i Etiopia

Institution resposible: Universitty of Bergen
Project manager: Bernt Lindtjørn

With reference to your application to the Regional Committee for Medical and Health Research Ethics, Western Norway (REK Vest), reviewed the application in meeting, 2014-08-14, pursuant to The Health Research Act.

About the project
The aims of the TB control programme are to reduce morbidity, mortality, transmission and to prevent drug resistance. Under Direct Observed Treatment Short course (DOTS), the research group rely on patients to present themselves to TB clinics for evaluation of their symptoms. Further goals are to evaluate the impact of DOTS expansion, identify the distribution and clustering of PTB+ and to determine the incidence of PTB+ cases.

The Committe`s considerations

Application/study protocol
REC Western Norway has no objections to the research questions, the purpose or the implementation of the project. The Committee sees this as a sound project, with the possibility for substantial benefits for the participants and the society.

Data security
Project end date is set to 29.09.2015. Identifiable personal data will be removed after the project has ended. Data must not be stored longer than necessary to complete the project and no longer than five years after the project has ended. The Committee sets as a condition that data is deleted or made anonymous as soon as it is no longer need for them, and no later than five years after the project has ended.

Conditions

- Data must be deleted or made anonymous as soon as it is no longer need for them, and no later than five years after the project has ended.
- The project must be approved by local authorities.

Decision

REC Western Norway approves the project in accordance with the submitted application as long as the aforementioned conditions are met.

Final Report and Amendments
The Project Manager shall submit a final report to the REC Western Norway according to Health Research Act § 12. The Project Manager shall submit an application of approval to REC Western Norway if there is significant changes in the project protocol, according to Health Research Act § 11.

Appeal
The Project Manager may appeal the committee's decision, see the Administration Act § 28. The appeal must be sent to the REC Western Norway within three weeks of receiving this letter. If the decision is upheld by REC Western Norway, the appeal will be forwarded to the National Research Ethics Committee for Medical and Health Research for a final assessment.

Med vennlig hilsen

Ansgar Berg
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The printed version of Paper III

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Prevalence and Incidence of Smear-Positive Pulmonary Tuberculosis in the Hetosa District of Arsi Zone, Oromia Regional State of Central Ethiopia

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Abstract

Background: The real burden of smear-positive (PTB+) and bacteriologically confirmed tuberculosis (BCTB) in Ethiopia is not known. Thus, the aim of this community-based study was to measure the prevalence and incidence of tuberculosis in the Hetosa District of Oromia Region, Ethiopia.

Methods: First, a population-based cross-sectional survey was conducted on a total of 33,073 individuals aged ≥ 15 years to determine the prevalence of PTB+ and BCTB cases. Then, in order to determine the incidence, a prospective follow-up was carried out on 32,800 individuals found to be either free from symptoms suggestive of TB (SSTB) during the baseline survey or had symptoms suggestive of TB but yielded negative bacteriological examination results. We identified 1,041 presumptive TB cases at the baseline survey, and 1,468 in the follow-up study. Each participants with cough of more than two weeks were provided spot and morning sputum samples for acid-fast bacilli sputum microscopy and culture.

Results: At the baseline survey, 43 BCTB cases were identified. Thirty six of these were both smear- and culture-positive while seven were only culture-positive. In the follow-up study, however, 76 BCTB cases were diagnosed and 70 of these were found to be both smear- and culture-positive while six were culture-positive only. The adjusted prevalence of PTB+ and BCTB in the study area was 109 and 132/100,000 persons, respectively. Moreover, the incidences of PTB+ and BCTB were 214 and 232/100,000 persons per year (py), respectively. The ratio of the passive to active case finding was 1:0.96 (45/43). For every TB case identified through the existing passive case diagnosis, there was an almost equal number (0.96) of undiagnosed infectious TB cases in the community. A family history of TB contact was independently associated with a high risk of TB (TB prevalence, AOR, 13; 95% CI: 6.55–15.33) and (TB incidence, aIRR 4.11, 95% CI: 2.18–7.77).

Conclusions and recommendations: The prevalence and incidence of smear-positive and bacteriologically confirmed TB cases were high in the study area. For every case of smear-positive TB receiving treatment, there was an almost equal (0.96) number of undetected infectious bacteriologically confirmed TB case in the community. The high proportion of undetected infectious TB cases in the community could possibly be due to the sub-optimal performance of Directly Observed Treatment Short-course (DOTS) in detecting 70% of infectious TB cases, as well as attaining a cure rate of 85% in the study area. Family history of TB contact has substantaially increased the risk of developing the disease, and there is a need to improve ways of identifying TB cases and intensify mechanisms of tracing contacts among household members of PTB+ cases.

Keywords: Pulmonary smear-positive TB, Prevalence, Incidence, Arsi Zone, Ethiopia

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Background

Even though highly effective first-line short-course regimens that can cure about 90% of tuberculosis (TB) cases have been available for decades, the disease remains a major cause of morbidity and the second leading cause of death worldwide. Only in 2014,there were an estimated of 9.6 million TB incidents and 1.5 million deaths due to the disease worldwide [1–3].

In 1993, the World Health Organization (WHO) declared TB to be a global public health emergency and in 1994 formally launched the Directly Observed Treatment Short-course (DOTS) as a standard strategy to control the disease [4]. Since then significant progress has been made in reversing the incidence of TB and it was possible to reduce its prevalence by 41% worldwide [5]. However, in sub-Saharan Africa and other resource-constrained countries, the number of new TB cases reported is steadily increasing. Moreover, 80% of TB cases and 78% of global TB deaths occur in these countries, primarily due to the high prevalence of human immuno-deficiency virus (HIV), poor TB control efforts, social inequalities, drug resistance and inadequate access to TB care [4–6].

The incidence and prevalence of TB are among the valuable epidemiological indicators used to measure the impact of TB control efforts and assess the progress made towards the Millennium Development Goals (MDGs) [7–9]. A recent systematic review has shown that the current fixed value of the annual risk of TB infection derived using the Styblo rule in the estimation of TB incidence in the community is no longer valid in the era of the HIV epidemic due to the fact that the incidence of TB cases is fuelled by the powerful interaction between tuberculosis and HIV [9, 10]. Hence, the true TB incidence and prevalence in the community could only be obtained through population-based surveys and prospective follow-up studies that measure the impact of TB control efforts in a particular country [8]. However, these types of data are lacking in developing countries including Ethiopia [11, 12].

Moreover, estimating the incidence of TB is a challenge due to the fact that enrolling many people in a prospective follow-up study is difficult [8]. As a result, two consecutive community-based prevalence surveys within a short time interval is an alternative option [8]. However, deriving TB incidence from prevalence surveys requires a good estimate of disease duration, which is difficult to obtain from such surveys. In general, the true TB incidence can be measured by either conducting a prospective follow-up study or by carrying out two consecutive prevalence surveys within a short time interval, and then estimating the number of new TB cases that occurs between the surveys [7–9].

According to the global TB report of 2015, the TB prevalence and incidence in Ethiopia were estimated at 190 (95% CI: 160–240) and 200 (95% CI: 160–240), respectively [1]. Moreover, the 2011 National TB Prevalence Survey and other reports from different parts of the country showed that the TB prevalence ranged between 30 and 213.4 per 100,000 population [12–18]. However, the real burden of smear-positive pulmonary TB and bacteriologically confirmed TB cases in Arsi Zone, in general, and Hetosa District, in particular, was not known. Thus, the aim of this study was to measure the prevalence of bacteriologically confirmed pulmonary TB at the baseline survey, and then to investigate the incidence of TB through a prospective follow-up study in the Hetosa District of Arsi Zone, Central Ethiopia.

Methods

Study setting

The Hetosa District is one of the 25 districts of Arsi Zone, Oromia Regional State of Central Ethiopia. The district is typical of the zone in terms of population density, socio–cultural and economic state, and demographic conditions. Therefore, it is expected that the results could be generalized to the whole zone. Based on the 2007 Census Projection, the district has an estimated population of 178,229 living in one urban and 23 rural *kebele*s (the smallest administrative unit in the government structure) where an average of 2.7 adults were living in each household [19]. Since 2010, each *kebele* in the district has been further divided into six sub-*kebele*s in the rural areas and 10 sub-*kebele*s in the urban centres. The sub-*kebele*s are known as *garee*s. The *garee*s of the district total to148 (138 from rural and 10 from urban *kebele*s) [20]. This study was carried out in 49 randomly selected *garee*s (clusters).

Study design and population

A population-based cross-sectional survey using multistage cluster sampling method was used to estimate the prevalence of smear-positive TB (PTB+) and bacteriologically confirmed TB (BCTB) cases at baseline. Next, a prospective follow-up study design was employed to estimate the incidence of the disease. In the estimation of PTB+ and BCTB incidence, individuals who were free of a persistent cough for more than two weeks, fever, loss of appetite, weight loss, blood-stained sputum and chest pain or difficulty in breathing [symptoms suggestive of TB (SSPTB)] at baseline, and those who had SSPTB at baseline but later showed negative result in a bacteriological test, were adopted as a cohort for the prospective follow-up study. The study was carried out from July 2013 to June 2014.

The source population for the study were adult individuals aged ≥15 years and permanently living in the district. Eligibility criteria were age ≥15 years, willingness to provide written consent to participate in the study, and a

permanent residence for at least 15 days in the selected house prior to the start of the study. Additionally, participants had to be individuals with SSPTB.

Sample size and sampling techniques

For economic and practical reasons, and because it is typical of the whole of Arsi Zone, the Hetosa District was purposefully selected from the 25 districts in the zone. All the 23 rural and one urban kebeles of the district were included in the study. The number of eligible population in the urban kebele was 5,903, and the population in the 23 rural kebeles was 27,170. Moreover, 18 of the rural kebeles have higher population density compared to the remaining five. Each of the former kebele has about double the population size of each of the latter. The number of clusters (garees) allocated to each urban and rural kebele was proportional to its population size. Consequently, a stratified multi-stage random sampling procedure was used to select two garees from each of the 18 rural kebeles high population density, and one garee from each of the remaining five rural kebeles. Moreover, based on the urban population of 5,903 and rural population of 27,170 that fulfilled the eligibility criteria, eight garees from the urban and 41 from the rural kebeles were included in the study [20]. An alphabetically arranged list of garees in each kebele along with their population size was obtained from the district authorities. Subsequently, garees were randomly selected from the list and included in the study. All individuals aged ≥15 years residing in the selected garees were included in the study.

The prevalence of PTB+ used in the sample size calculation was 382 per 100,000 based on the assumption that has been used in the 2011 national prevalence survey of the adult population aged ≥15 years [21], and an estimated 210/100,000 in 2012 for Ethiopia by the WHO [22]. Consequently, we calculated the sample size using a prevalence of 382 per 100,000, a relative precision of 0.25, an expected participation rate of 90% and a design effect of 2, a sample size of 33,448 people from 49 clusters. However, in the house-to-house enumeration held during the pre-survey of all the 49 selected *garees*, 34,707 adults aged ≥ 15 years were identified. As the number was very similar to that of the calculated sample size, we enrolled all of them in the study.

Data collection procedures

The aims of the study and the procedures for data collection were discussed with zonal, district and *kebele* leaders. The District TB Coordinator selected 24 nurses and 24 health extension workers (HEWs) for data collection, five laboratory technicians for sputum sample collection and 10 health officers for supervision. A health extension worker (HEW) is a female community health worker trained for one-year and deployed at a *kebele* with the responsibility of providing essential health services to ensure equitable access to health care, prevent major communicable diseases and promote health in the community [23]. Altogether, a total of 48 data collectors, five laboratory technicians and 10 supervisors were trained on TB screening techniques and on how to collect and transport sputum specimens. As on average about 2.7 adult were living in each household, each data collector was responsible to interview for about 11 to 12 study participants from four to five households with in a day.

The baseline survey to determine the prevalence of PTB+ and BCTB was conducted in May to June 2013. Subsequently, the prospective follow-up study to determine the incidence of PTB+ and BCTB was carried out between July 2013 and June 2014. Individuals who met the eligibility criteria, and were willing to provide written consent to participate in the study were included.

Study participants with SSPTB were identified as presumptive TB cases and were interviewed about their age, sex, history of contact with known TB patients and any current or previous TB treatment both at the baseline survey and prospective follow-up study. Participants with any SSPTB were requested to submit two sputum samples, one on the spot and the other in the morning of the following day. Upon receipt from the participants, the specimens were immediately put in sterile flacon tubes and placed in a cold box at 4°C and transported on the same day to the Adama Regional Research Centre Laboratory.

The following day each smear was fixed, air-dried and stained using the standard Ziehl-Neelsen (ZN) methods [24] and examined by experienced laboratory technicians for the presence of acid-fast bacilli (AFB). Positive results were quantified using the International Union against Tuberculosis and Lung Disease (IUATLD) standards [25]. A senior laboratory technologist blinded to the first test results re-examined all the smear-positive and 10% of the smear-negative slides. However, no discordant test results were observed between the two examinations. Moreover, sputum cultures using Lowenstein-Jensen (LJ) medium were commenced within a maximum of two days from the receipt of the sputum. In the event the diagnostic test did not commence on the 2nd day following specimen collection, the sputum sample was stored at −20°C in the same laboratory until tests were undertaken.

The data collectors also checked TB patient registration units in the study area to verify that those who reported they were on anti-TB treatments at the time of the study were actually on medication, and if there was any patient who was on anti-TB medication but did not report it during the survey period. However, no mismatch was identified.

Pulmonary smear-positive TB (PTB+) is defined as a patient found to be positive for AFB in both spot and morning sputum samples examined using direct microscopy or a patient found to be smear-positive in either spot or morning sputum examinations for AFB and culture-positive. Further, bacteriologically confirmed TB (BCTB) cases are individuals with smear- and/or culture-positive results. Types of TB were defined based on the 2011 WHO Tuberculosis Prevalence Survey Handbook [26] as follows:

New case not on treatment

A patient who has never received TB treatment for more than a month and who is not being treated currently with any anti-TB drugs.

New case on treatment

A patient who is currently being treated with anti-TB drugs, but has previously not received any anti-TB treatment for more than a month.

Previously treated case not on treatment

A patient who has previously received treatment for TB for more than a month, but who is currently not receiving any treatment with anti-TB drugs.

Previously treated case on treatment

A patient who has previously received treatment for TB for more than a month and who is currently being treated with anti-TB drugs.

The sputum smear-positive results were communicated through both written and telephone reports to TB focal persons at health centres in the study sites. The diagnosed TB cases started anti-TB treatment according to the national TB guidelines [24], with a culture performed on morning specimens using Lowenstein-Jensen (LJ) medium. The results were considered to be negative if no colonies were identified after eight weeks of incubation. Positive results from the LJ cultures were confirmed by testing for the presence of AFB through microscopic examination using the Ziehl-Neelsen method.

In the follow-up study, a total of 32,800 individuals who were free from SSPTB at baseline study and those with SSPTB but negative bacteriological results during the same survey were followed up for 12 months (July 2013 to June 2014) so as to estimate the incidence of PTB+ and BCTB cases. At intervals of six months, both at the end of the sixth and the 12th months from the baseline study, the same data collectors revisited all households that had been visited at the baseline and interviewed each person aged ≥15 years. The same data collection procedure, sputum sample collection, laboratory testing procedures and questionnaire were used in the prospective follow-up study. To ensure the data quality, the principal investigator and supervisors closely monitored the data collection process.

Data entry and analysis

All data collected using the standardized and pre-tested questionnaire were coded and double-entered into Epi-info version 7 statistical software. The data were checked against the original questionnaires for missing variables, and errors were corrected by referring to the original questionnaires. Data analyses were performed based on the method recommended by the WHO Tuberculosis Prevalence Survey Handbook for the estimation of PTB+ and BCTB prevalence [26]. In the initial model of analysis, the crude PTB+ and BCTB prevalence was estimated without taking into account the sample cluster survey design effect. Nonetheless, in the final model, a complete analysis with an inverse probability weighting was carried out using robust standard errors to account for the sample cluster survey design effect, and the adjusted estimated prevalence of both PTB+ and BCTB were computed and reported [26]. Data analysis was carried out using STATA (v12.1, Stata Corporation, College Station, TX, USA).

Furthermore, in the analysis of TB incidence, a persons per year observation (pyo) was used as a denominator where person-time at risk of TB began in June 2013 when eligible individuals started participating in the study. Enrolment ended when participants were found to be AFB and/or culture-positive and were censored in June 2014. However, as the exact time of contribution of those who dropped out in the course of the study due to out-migration or death was not known, we excluded 185 participants (17 deaths, 47 refusal cases and 119 out-migrants) from the analysis to avoid bias due to ambiguity surrounding the time of their contribution. In order to avoid an under estimation of TB incidence, the principal investigator checked all health facilities in the study area to verify whether any TB cases were diagnosed and registered during the 12 months of the follow-up study, but none was found.

The prevalence and incidence of PTB+ and BCTB were taken as the dependent variables whereas age, sex, area of residence and family history of TB contact were the independent variables. The independent and dependent variables were further categorized into groups for analysis. A Poisson regression analysis was carried out in the analysis of TB incidence. The estimated incidence rate ratios (IRRs) and adjusted odds ratio (AOR) at 95% confidence intervals (CI) and *P*-values of less than 0.05 were used to assess the strength of association with PTB+ and BCTB cases as the outcome.

Ethical considerations

The study protocol was reviewed and approved by both the Regional Committee for Medical and Health Research

Ethics in Western Norway (REK Vest) and the Institutional Review Board Committee at the Oromia Health Bureau, Ethiopia. All participants were informed that taking part in the study was based fully on their willingness, and that they had the right to quit at any time from the study. Before any interview started, in the prevalence survey and in the subsequent follow-up study written consents were obtained from all participants aged ≥18 years and from parents/guardians if participants were <18 years of age. Data on individuals were analysed and anonymously reported. Immediate referrals were arranged for participants found to be smear or culture-positive, and all started anti-TB treatment at health centres close to them. The principal investigator also confirmed that all patients started treatment.

Results

Survey population

A total of 63,312 individuals were enumerated during the pre-survey census (Fig. 1). Of these, 34,707 were eligible and thus participated in the prevalence survey. Of participated, 33,073 (95.3%) were screened for SSPTB. The average number of eligible individuals who participated from each cluster was 674.9. The mean age of the screened individuals was 33.3 years (standard deviation; SD 16.2) and the median age was 30.3 years. The overall response rate was 95.3%, with 95.8% for females and 94.8% for males. The overall participation rate was over the 90% expected in the study design. However, a higher participation rate among rural clusters (97.6%) was seen compared to the urban ones (85.9%). Out of the total of 34,707 eligible individuals, 1,634 (4.7%) did not participate in the prevalence survey. Of the latter, 1,489 (91.1%) were not at home, while 145 (8.9%) were not willing to participate in the survey (Table 1 and Fig. 1).

Screening and sputum submission

A total of 33,073 eligible individuals were screened for SSPTB in the prevalence survey. Of these, 27,173 (82%) were rural residents and 16,907 (51.1%) were males. Moreover, a total of 32,800 individuals were enrolled for the follow-up study (Table 1). Of these, 31,802 were free of SSPTB at the baseline survey, while 998 showed SSPTB but yielded a bacteriologically negative result. A total of 1,041 and 1,468 individuals at the baseline survey and during the follow-up study, respectively, were reported to have SSPTB. These provided two sputum samples for bacteriological examination (AFB microscopic examination and culture). Of the former group, 258 (24.8%) individuals reported previous history of TB treatment, but were not on anti-TB treatment at the time of the survey. However, 45 known PTB+ cases were diagnosed through passive TB case findings and had been on anti-TB treatment at the time of the study. These also provided two sputum samples for bacteriological examination. Fig. 2 summarizes the screening and subsequent bacteriological examination results.

TB cases identified

A total of 1,041 individuals with SSPTB and 45 PTB+ cases known to be on anti-TB treatment at the baseline survey provided spot and morning sputum samples for bacteriological examination. Of the 1,041 presumptive TB cases, 43 were found to have bacteriologically confirmed TB (culture and/or smear positive) whereas none of the 45 individuals on anti-TB treatment showed a positive result (Fig. 1).

The mean (SD) age of the diagnosed TB cases was 30 (10.3) years and the median was 30.8 years. Of the 43 bacteriologically confirmed TB cases, 36 were both smear- and culture-positive while seven were smear-negative but culture-positive. From the 36 smear-positive TB cases, 12 (33.3%) had a family history of TB contacts (Table 2).

Furthermore, 32,800 individuals were enrolled in the follow-up study. Of these, 32,759 were followed for 12 months, making 393,108 person-months of observation, and 41 were diagnosed with TB at the end of the sixth month making 246 person-months of observation. Overall, a total of 393,354 person-months or 32,779.5 person-years (py) were observed. Of the total of 393,354 person-months or 32,779.5 py observed, 76 bacteriologically confirmed TB cases were identified (41 at the end of the sixth month and 35 at the end of the 12th month). Of these, 70 were smear- and culture-positive while the remaining six were smear-negative but culture-positive (Fig. 1 and Table 3).

Prevalence

The adjusted prevalence estimate of PTB+ individuals among adults aged ≥15 years was 109 (95% CI: 67.2–150.5) whereas that of BCTB was 132 (95% CI: 83.0–176.2) per 100,000 population. Even so, the adjusted prevalence of BCTB was higher among females (148.5) (95% CI: 81.1–210.4) than males (112.2) (95% CI: 55.8–170.2) but had no statistical difference (Table 2).

In the multivariate logistic regression model, age and family history of TB contacts were independently associated with high rates of PTB+ and BCTB cases. Compared to individuals in the age group from 15–24 years, those in the age group from 25–34 years were 3.4 times more likely to have TB [AOR: 3.4 (95% CI: 1.4–8.6)]. Those in the age group of 35–44 years were 4.2 times more likely [AOR: 4.2 (95% CI: 1.7–10.2)] while those ≥45 years were 2.7 times more likely to have the disease [AOR: 2.7 (95% CI: 1.1–6.7)]. The prevalence of TB therefore increased with age up to 44 years but declined from 45 years onward. Presumptive TB for those

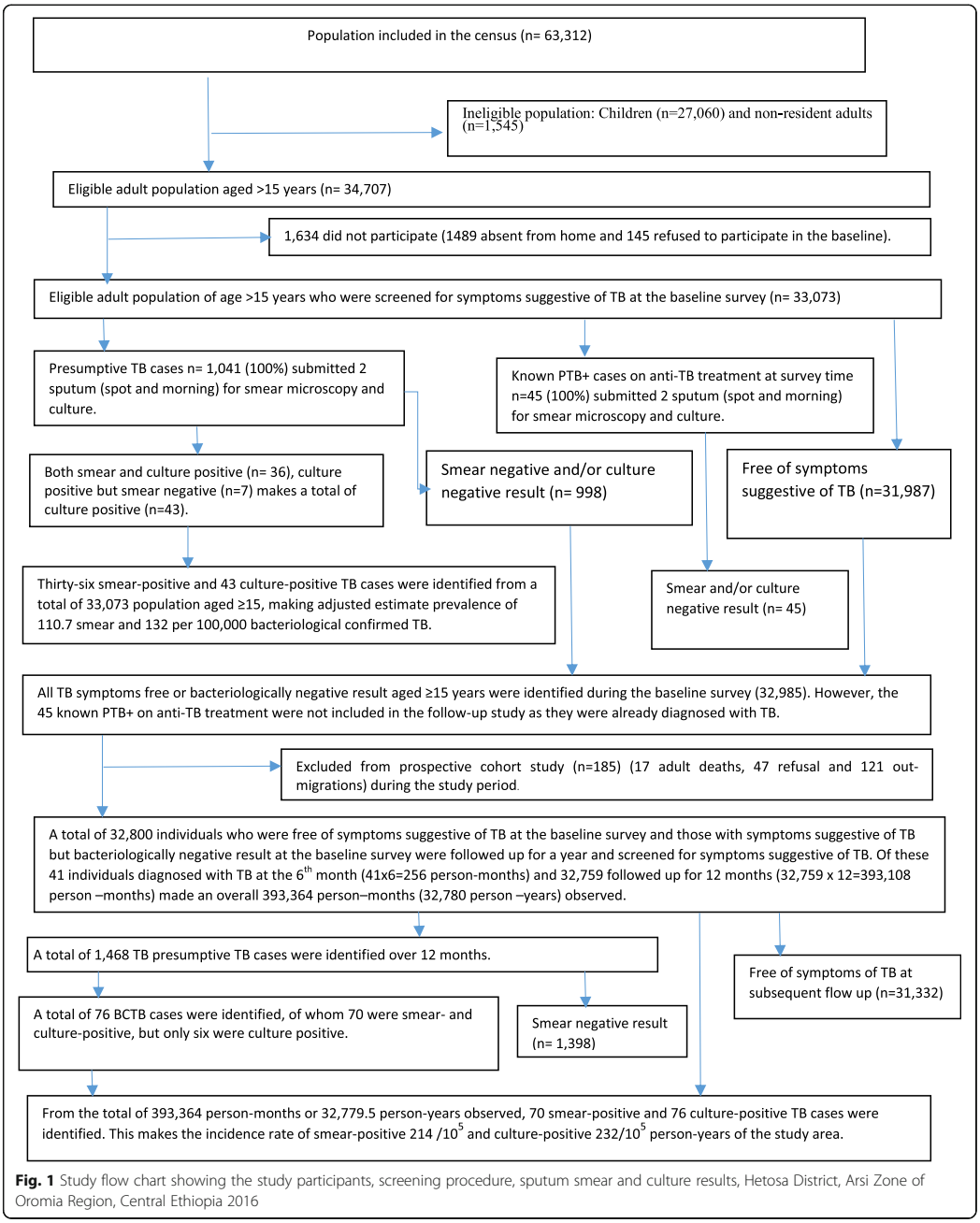

Fig. 1 Study flow chart showing the study participants, screening procedure, sputum smear and culture results, Hetosa District, Arsi Zone of Oromia Region, Central Ethiopia 2016

Table 1 Participation of eligible individuals in the prevalence survey, Hetosa District of the Arsi Zone of Oromia Region, Central Ethiopia

Variable	No. of eligible individuals identified through census and expected to participate	No. of eligible individuals who participated in the survey and were screened	Proportion of screened participants from the total eligible population
Sex			
Male	17,834	16,907	94.8
Female	16,873	16,166	95.8
Total	34,707	33,073	95.2
Age			
15–24	12,115	11,691	96.5
25–34	8,331	7,889	94.7
35–44	5,959	5,697	95.6
≥45	8,302	7,796	93.9
Mean age	33.3 (SD 16)		
Total	34,707	33,073	95.2
Stratum			
Rural	27,838	27,170	97.6
Urban	6,869	5,903	85.9
Total	34,707	33,073	95.2
PTB+ identified through passive case findings			
Male		28	Male to Female ratio
Female		17	
Total		45	1: 0.61
Number of BCTC identified by the survey			
Male		19	Male to Female ratio
Female		24	
Total		43	0.79:1

who had a family history of contact with TB patients was 13 times more likely than those without such a history [OR = 13.0, (6.5–25.3)].

The active and passive TB case findings of the study area were compared using the number of cases identified by each method. Forty-three BCTB cases were identified through current active TB case findings while 45 PTB+ cases on anti-TB treatment at the time of the survey were detected through passive case findings. Of the 45 PTB+ cases, 28 were males while 17 were females making a ratio of 1:0.61 (28/17). The male to female ratio for those identified through active case findings was 0.79:1 (19/24). The ratio of passive to active case findings was 1:0.96 (45/43) (Table 1).

Incidence of smear-positive TB

From the total of 393,354 person-months (32,779.5 py) observed, 76 BCTB cases were identified. Of these, 70 were both smear- and culture-positive, while six were smear-negative but culture-positive. The incidence rate of PTB+ among adult individuals aged ≥15 years was 214 (95% CI: 163.5–263.5) whereas that of BCTB was 232 (95% CI: 179.7–283.9)/100,000 py in the study area (Table 3). There were 17 adult deaths, 47 refusals and 121 out-migrations during the study period and these were excluded from the analysis. Moreover, because the TB status of 1,634 individuals who did not participate in the baseline survey was not known, they were also excluded from the follow-up study (Fig. 1).

The incidence of PTB+ cases among males was 215 (95% CI: 144.8–285.3) whereas it was 212 (95% CI: 140.7–283.2) among females per 100,000 py. Likewise, the incidence of PTB+ per 100,000 py among urban residents was 222 (95% CI: 101.5–343.4) while it was 212 (95% CI: 156.7–266.5) among rural people. However, the difference in the incidence rates among males and females (adjusted incidence rate ratio) [aIRR, 1.22 (95% CI: 0.78–1.93], and among urban and rural dwellers [aIRR, 1.23 (95% CI: 0.71–2.14)] was not statistically significant (Table 3).

In the multivariate Poisson regression model, the age and history of TB contact were independently associated with a high risk of TB. Presumptive TB cases in the age group from 35–44 years were 2.4 times [aIRR, 2.40 (95% CI: 1.18–4.55)] more likely to have TB compared to those in the younger age group from 15–24 years.

Table 2 Prevalence of smear-positive and bacteriologically-confirmed pulmonary TB among population aged ≥15 years, Hetosa District of Arsi Zone of Oromia Region, Central Ethiopia

Category	Number of participants	Smear-positive Pulmonary Tuberculosis (PTB+)			Bacteriologically Confirmed Pulmonary Tuberculosis (BCTB)			
		Number of PTB+	Crude Prevalence estimate of PTB+ (95% CI)/100,000	Adjusted* prevalence estimate of PTB+ (95% CI)/100,000	Number of BCTB	Crude Prevalence Estimate of BCTB (95% CI)/100,000	Adjusted* Prevalence estimated of BCTB (95% CI)/100,000	Adjusted Odds Ratio
sex								
Male	16,907	16	89 (48.3–141.0)	89 (42.2–147.1)	19	112 (62.0–163)	112.2 (55.8–170.2.)	1.00
Female	16,166	20	124 (69.5–177.9)	124 (63.4–184.0)	24	149 (89.1–215.0)	148.5 (81.9–210.4)	1.3 (0.71–2.43)
Age								
15–24	11,691	5	43 (5.3–80.3)	43 (4.1–81.3)	8	68 (26.3–92.2)	68 (19.1–99.4)	1.00
25–34	7,889	12	152 (66.1–238.1)	152 (60.0–244.2)	13	165 (75.2–254.0)	165 (68.0–261.2)	3.4 (1.38–8.61)
35–44	5,697	11	193 (79.1–307.1)	193 (73.0–313.2)	11	193 (79.1–307.2)	193 (72.0–314.4)	4.2 (1.71–10.20)
≥45	7,796	8	103 (31.5–237.7)	103 (25.4–233.8)	11	141 (57.8–224.4)	141 (50.6–231.6)	2.76 (1.14–6.72)
Residence								
Urban	5,903	5	85 (10.5–159)	85 (4.4–165.1)	9	153 (104.2–254.0)	153 (97.0–261.2)	1,23 (0.59–2.57)
Rural	27,170	31	114 (73.9–154.2)	114 (67.5–160.3)	34	125 (97.3–224.4)	125 (90.1–231.6)	1.00
History of TB contact								
NO	31,170	24	77 (42.0–107.7)	77 (36.0–113.8)	31	98 (65.2–133.8)	98 (58.0–141.0)	1.00
Yes	1,867	12	643 (280.2–1005.2)	894 (274.1–1011.3)	12	643 (280.2–1005.2)	643 (273.0–1012.4)	13.0 (6.55–15.33)
Total	33,037	36	109 (73.3–144.3)	109 (67.2–150.5)	43	130.2 (91.2–169)	130.2 (83–176.2)	

NB. *Adjusted prevalence estimate of PTB+ and BCTB analysed using robust standard errors to account for the sample survey design

Table 3 Study population, smear-positive TB cases identified over 12 months and incidence rate per 100,000 persons per year, Hetosa District, Arsi Zone of Oromia Region, Central Ethiopia

Category	Person-year	Smear-positive Pulmonary Tuberculosis (PTB+) ($n = 70$)			Bacteriologically Confirmed Pulmonary Tuberculosis (BCTB) ($n = 76$)		
		Number of diagnosed PTB+ cases	Incidence rate per 100,000 person-year (95% CI)	Adjusted Incidence Rate Ratio (aIRR) (95% CI)	Number of BCTB	Incidence rate per 100,000 person-year (95% CI)	Adjusted Incidence Rate Ratio (aIRR) (95% CI)
sex							
Male	16,741	36	215 (144.8–285.3)	1.00	38	227 (156.7–299.2)	1.00
Female	16,038.5	34	212 (140.7–283.2)	1.22 (0.78–1.93)	38	237 (161.6–312.3)	1.26 (0.80–1.97)
Age							
15–24	11,631.5	16	138 (70.2–205)	1.00	19	163(64.1–236.8)	1.00
25–34	7,865	8	102 (31.2–172.2)	1.30 (0.64–2.67)	9	114 (39.7–189.2)	1.51(0.73–3.15)
35–44	5,514	20	363 (203.7–521.7)	2.40 (1.18–4.55)	21	381(218.0–543.7)	2.76 (1.38–5.52)
≥45	7,769	26	335 (206.0–463.3)	1.66 (1.44–4.91)	27	348 (216.4–478.6)	3.05 (1.63–5.71)
Residence							
Urban	5,843.5	13	222 (101.5–343.4)	1.00	16	274(139.6–408.0)	1.00
Rural	26,936	57	212 (156.7–266.5)	1.23 (0.71–2.14)	60	223(166.4–279.10	1.25 (0.72–2.16)
History of TB contact							
NO	31,138	50	161 (116.1–205.1)	1.00	56	180(132.7–217.6)	1.00
Yes	1,641.5	20	1218 (686.4–1752.4)	4.11 (2.18–7.77)	20	1218(686.4–1752.4)	5.11 (2.63–9.96)
Total	32,779.5	70	214 (163.5–263.5)		76	232(179.7–283.9)	

Compared to the same age group, those aged ≥45 were 2.7 times [aIRR 2.66 (95% CI: 1.44–4.91)] more likely to develop the disease. Presumptive TB cases who were either free of SSPTB or showed negative bacteriological examination results at the baseline survey but had history of contact with TB patients in the family were four times more likely to have TB than those with no history of such contact [aIRR, 4.11 (95% CI: 2.18–7.77)] (Table 3).

Discussion

This population-based study identified a high prevalence and incidence of PTB+ and BCTB among individuals aged ≥15 in Hetosa District of Arsi Zone. For every TB case of PTB+ on treatment, there was an almost equal number (0.96) of undetected bacteriologically confirmed infectious TB cases in the community. The overall crude prevalence point estimate of PTB+ and BCTB cases was very similar to the inverse probability weighting prevalence point estimate using robust standard errors to account for the cluster survey sample design effect. Even so, there was a difference in precision with a wide confidence interval for the adjusted prevalence point estimate. The adjusted prevalence estimate of PTB+ and BCTB cases in the study area was 109 (95% CI: 67.2–150.5) and 130.2 (95% CI: 83.0–176.2)/100,000 population, respectively. In the follow-up study, the incidence of PTB+ and BCTB was 214 (95% CI: 163.3–263.5) and 232 (95% CI: 179.7–283.9)/100,000 py.

The 130.2/100,000 adjusted prevalence estimate of BCTB cases identified in this study is higher than the 34/100,000 reported from China and the 76/100,000 from Southwest Ethiopia [12]. Nonetheless, it is lower than the 169/100,000 report from Northern Ethiopia [27] and the 278/100,000 from the Lao PDR [28].

Moreover, the 109/100,000 adjusted prevalence of PTB + cases in this study is similar to the 108/100,000 report of the national estimate [29]. Conversely, it is higher than previous reports that ranged from 30 to 80/100,000 population in different parts of the country [12, 14, 30], the 90/100,000 from Eritrea [31] and the 95/100,000 from Bangladesh [32]. Still, it is lower than the145/100,000 population reported from Vietnam [33], and the 169/100,000 population reported from Northern Ethiopia [27] and India [34].

The difference in the prevalence of BCTB and PTB+ TB cases across different geographic settings might be due to differences in the populations studied, the timing of the study or differences in the sampling, data collection and screening methods used across the different studies. For example, in some studies [12, 14, 30] the heads of households were interviewed to give testimony about the TB symptomatic cases of all family members in the household. However, the heads of households may not have sufficient information about all individual members while others interviewed all members of a household to screen presumptive TB cases [13, 29].

Some community-based TB prevalence studies used clinical diagnoses and chest x-rays before taking sputum for screening [29, 35, 36] while others, including the current study, used TB symptom-screening questionnaires [12–14, 36] to identify the cases. Nonetheless, the chances of detecting TB cases among non-symptomatic individuals increased by 20–50% when a combination of a TB symptom-screening questionnaire and a clinical diagnosis with a chest x-ray was employed, compared to using a TB symptom-screening questionnaire alone without a chest x-ray [7, 28, 29, 35, 37]. Hence, the adjusted estimate of BCTB prevalence in this study might be underestimated due to the fact that the chest x-ray screening method was not used to identify non-symptomatic TB cases.

The age group in the survey also varied across different studies. Some covered all age groups ≥15 years [29, 30, 32, 38] while others included those aged >14 years [14] and still others those aged 14 years [12, 13]. Consequently, a comparison of TB prevalence rates among studies within a country or elsewhere should be taken with caution.

The high prevalence of BCTB cases among younger age groups in this study is in agreement with a previous report [29], whereas the high prevalence of TB among the younger population may suggest ongoing TB transmission in the community. The prevalence of TB increased with age among the younger age groups up to the age of 45 years. However, a high TB incidence rate was observed among the older age group. This might be due to the high number of infectious TB cases identified at baseline, which could reduce the ongoing TB transmission among the general population, while the high TB incidence among the elderly is probably indicative of a latent TB reactivation among the older age group [13, 14, 29, 39]. Nevertheless, further study is required to fully understand why the observed high TB prevalence among younger individuals also corresponded to a high incidence in the older age group.

In this study, 43 BCTB cases were identified through active TB case findings at the baseline survey while 45 PTB+ cases were identified through existing passive TB case findings. Thus, the ratio of PTB+ cases being treated at the time of the survey to newly detected BCTB cases was 1:0.96 suggesting that for every PTB+ case receiving treatment during the survey, there was an almost equal number (0.96) of cases of BCTB existing in the community. This indicated that there was a very high proportion of undiagnosed infectious TB cases present in the community. In Southern Ethiopia, there were two cases [14] while in South Africa there were 4.5 cases [40] of passive detection for every TB case

identified through active case findings. In Northern Ethiopia, the ratios of passive to active TB case findings were 2.5:1 [30] and 2:1 [13]. This implies that there is a high number of undiagnosed infectious TB cases in the present study area compared to reports by previous studies. The high proportion of undetected infectious TB cases in the community might be due to the sub-optimal DOTS performance in identifying 70% of infectious TB cases and attaining the global target of 85% cure rate in Ethiopia [41–43].

Moreover, the difference in the number of undetected infectious TB cases across different geographic settings might be attributed to variation in DOTS performance, DOTS service coverage and the quality of DOTS services across various study areas. It could also be attributed to the difference in DOTS service uptake that might result from differences in public awareness about TB. Consequently, the decentralization and strengthening of the community in TB care could help to pick up undetected infectious TB cases in Arsi Zone.

The male-to-female ratio among PTB+ cases identified through existing passive TB case findings was 1:0.61 (28/17) whereas the ratio among BCTB cases identified by the current active TB case finding was 0.79:1 (19/24). This may indicate a lower rate of passive case findings among females compared to males. The lower passive and higher active TB case findings among women in this study is in agreement with reports from Southern [14] and Northern Ethiopia [13, 27], Bangladesh [44] and India [34] where more women were identified through active TB case findings. The lower passive TB case findings among females might be due to poor access to health services, and as shown in a study conducted in South Africa, women are less likely to be asked for a sputum sample when they appear at health facilities [45]. Moreover, their economic dependence and low health-care seeking behaviour possibly hindered women from visiting health institutions to obtain TB care services. Barriers to accessing health services among TB patients and a failure to detect women with TB through the routine TB control programme warrant further inquiry.

As expected, history of TB contact increased the risk of having active TB. A recent systematic review and previous reports have shown that history of TB contact was associated with a high risk of TB [30, 46–48]. Findings by the current study are in line with those of a systematic review and large epidemiological surveys that have established the association between history of TB contact and higher risk of TB [47, 49, 50]. Therefore, contact-tracing efforts should target households with members who are PTB+ so as to capture the undetected infectious TB cases in the community.

In this study, the high prevalence of BCTB cases in urban areas confirms previous reports of high TB prevalence in urban settings [16, 51, 52]. In contrast, the national prevalence TB survey reported higher TB prevalence among dwellers in the rural areas [29]. This is due to the inclusion in the national prevalence survey of pastoralists in the rural population where the highest prevalence ratio of 170/100,000 was observed [21] as opposed to the current study. The pastoralist population may have poor access to TB care, as well as low awareness and health-seeking behaviour which might have resulted in them having a high burden of undiagnosed TB cases and eventually elevating the prevalence of TB among the rural population in the national prevalence survey. The higher prevalence of BCTB cases among urban settings compared to rural areas in the current study may be due to the overcrowded living conditions and dichotomy of higher HIV prevalence in the urban areas of the country.

The 214/100,000 py incidence rate of PTB+ cases in this study is similar to the 212/100,000 py reported from South Africa [53]. However, it is higher than the 197/100,000 py from Guinea-Bissau [54] and the 207/100,000 py from Southern Ethiopia [15]. Nonetheless, it is lower than the 311/100,000 py reported from Northern Ethiopia [11]. The high incidence of TB in the present study might be an indication of the ongoing transmission of the disease that might result from a sub-optimal DOTS performance in the interruption of TB transmission. For instance, according to previous reports, there was a low rate of PTB + case detection rate (37.7%) [41] and a low cure rate (66.9%) [42] and a high prevalence of drug resistance TB [55] in the study area. Hence, the low PTB+ case detection rate, cure rates and high drug resistance TB reported from the study area, combined with the high prevalence and incidence rates identified by the current study, may confirm the sub-optimal performance of DOTS in curbing the active transmission of TB. Therefore, the involvement of health extension workers in educating the community on TB as well as accelerating referral of presumptive TB cases may improve the possibility of capturing undiagnosed infectious TB cases in the community.

Information on the prevalence and incidence of TB is a valuable epidemiological indicator to help assess the impact of national and international TB control efforts. Nevertheless, community-based data on BCTB prevalence and incidence are lacking in developing countries including Ethiopia. As a result, the findings of this study with regard to the prevalence and incidence rates of BCTB cases are among the very few population-based studies in resource-poor settings.

In this study, efforts were made to maintain the quality of the study, and rigorous training was given for data collectors and laboratory technicians. The study population was monitored to identify deaths and migrations during the prospective follow-up study to provide an

accurate time contribution in the denominator to compute the incidence rate. Moreover, we have used very sensitive standardized and pre-tested questionnaires to screen presumptive TB cases experienced and qualified laboratory technicians to carry out smear microscopy and sputum culture. Following that, a senior laboratory technologist who was blinded to the results of the first test results re-examined all the smear-positive, and 10% of the smear-negative slides, to validate the quality of laboratory results. Additionally, the estimated design effect we have used in this study was 2 whereas the actual calculated design effect from the current study data was 1.3 there by indicating that the sample size of our study was adequate and is representative of the study population of the district. Likewise, in order to obtain the adjusted precision of PTB+ and BCTB prevalence of the study, the design effect for the cluster sample survey was taken into account during the analysis.

On the other hand, although our study was among the very few attempts to detect a community-based TB incidence and may contribute valuable information to the TB control programme in Ethiopia, it has some limitations. First, SSPTB was used as screening mechanism. The fact that chest x-ray was not used in our study might underestimate the prevalence and incidence of TB in the area. The missing diagnosed TB cases at the baseline survey but which were later included in the prospective study might have resulted in an over-estimation of the TB incidence rate. Second, we excluded 20 contaminated sputum cultures from the analysis at the baseline survey and this may also have led to an underestimation of the prevalence of sputum culture TB. Third, we carried out a survey three times, first at the beginning of the study to determine the prevalence of TB, followed by the second at the end of the sixth month and the third at the end of the 12th month to estimate incidence of TB. However, the six-month time interval between surveys may have given sufficient time for spontaneous self-cure of active TB cases, which might have led to underestimation of the true incidence of TB cases in the study area. Fourth, we excluded 1,634 individuals who did not participate in the baseline survey from the subsequent follow-up study. Moreover, 47 individuals who refused to participate in the follow-up study plus 18 deaths and 119 out-migrants were excluded from the analysis of the incidence rate due to the fact that the exact time of their contribution to the denominator was not known. Nonetheless, the overall proportion of participants excluded was only 5.5% and their baseline socio-demographic characteristics were similar to those included in the analysis. Therefore, their exclusion may not affect the overall findings of the study. Fifth, although HIV is a known risk factor for TB, we did not screen presumptive TB cases for HIV to measure the impact of HIV in fueling TB in the study area. Sixth, some relevant variables that might have affected the outcomes of interest were not included in the study. Hence, stratifying and analyzing only those included variables is less likely to fully control for other possible confounding variables and may introduce bias.

Conclusions and recommendations

The prevalence and incidence of smear-positive and bacteriologically confirmed TB cases were high in the study area. For every case of smear-positive TB receiving treatment, there was an almost equal number (0.96) of undetected infectious bacteriologically confirmed TB cases in the community. The high proportion of undetected infectious TB cases in the community could have resulted from the sub-optimal DOTS performance in detecting 70% of infectious TB cases and attaining a cure rate of 85% in the study area. For this reason, there is a need to design an alternative strategy to improve TB case findings. A family history of contact has substantially increased the risk of developing the disease, so there is a need to improve the identification of TB cases and intensify contact tracing among household members of PTB+ cases through the involvement of community-based health extension workers.

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Availability of data and materials

The dataset supporting the conclusions of this article can be made freely available to any scientist wishing to use them for non-commercial purposes.

Authors' contributions

SDH was the principal investigator responsible for designing and conducting the study and was involved in analysing the data and writing the manuscript. BL participated in designing the study, analysing the data and writing the manuscript. MD participated in designing and writing the manuscript. DT participated in the data collection and analysis. MS participated in the laboratory work. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics and consent to participate

An official letter of ethical approval by the Institutional Review Board Committee at the Oromia Regional Health Bureau, Ethiopia, and subsequently by the Regional Committee for Medical and Health Research Ethics in Western Norway (REK Vest), and written consents obtained from participants were filed and kept at the Oromia Regional Health Bureau and can be presented for verification at any time.

Declarations
The authors declare the ethics and consent to participate, the consent to publish, competing interests and the availability of data as follows.

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