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M. *tuberculosis* among jail inmates of Ethiopian prisons: risk factors, molecular epidemiology and drug resistance

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Contents

Abstract of dissertation

Background: In the 21st century the advancements of science have broaden our understanding about the pathology, treatment, drug resistance, evolutional phylogeny, population structure and transmission dynamics of the TB bacilli. However, these advancements were not sufficient enough to halt the TB epidemic in many Sub Saharan countries. Especially, the emergence of multi drug resistance tuberculosis has posed a significant threat to global TB control. In Ethiopia, TB is one of the three top killer infectious diseases. It is still the major problem in some pocket geographical areas, refugee camps and prisons. TB in prison was not receiving enough attention in the past years, considering the role prisons are playing in ongoing local TB epidemics. As a result, the burden of TB in Ethiopian prisons was largely obscured.

Objective: To determine the epidemiology and risk factors of TB in prisons, together with the population structure, transmission dynamics and drug resistance profile of *Mycobacterium tuberculosis complex* isolates in Ethiopian prisons.

Methodology: A two phases cross sectional study was done between Jan, 2013 and May, 2015. Prisons and communities living in south western, southern and Eastern part of the country were included. In the first phase 13 zonal prisons from Oromia, SNNPRS and Harari were included to determine the magnitude and identify the risk factors for TB in prison. In phase two, all *M.tuberculosis* complex (MTBC) strains isolated in prison and additional 106 control MTBC strains collected from newly diagnosed smear positive TB patients attending selected hospitals at regional states of Oromia, South Nations and Nationalities Peoples, Harari, Somali and Dire Dawa city administration were included.

Result: A total of 15,495 inmates were screened by WHO TB screening criteria and 765 suspects were identified. The prevalence of tuberculosis in Ethiopian prison was 458/100.000 inmates. Alcohol consumption, contact with TB patients at home, window availability in prison cells had contributed for the observed prevalence. Furthermore, a total of 11 different lineages/ sub-lineages were identified by combined technique of MIRU-VNTR and spoligotyping. The clustering rate of isolates from prison and community was 28.57% and 31.82% respectively, with some strains from prison and communities sharing the same cluster. The predominant genotype was the recently described Ethiopian_H37Rv like with equal distribution between the prison and the community isolates. The MDR prevalence in the community was 2.27% with no difference with that of prison.

Conclusion: The prevalence of tuberculosis in Ethiopian prisons is more than twice higher than the population estimate. The diverse population structure and low clustering of MTBC observed in this study has indicated that most of the TB cases in prison and communities were resulted from reactivation of remote infection. The magnitude of MDR in prison as well as community is

very worrisome. Hence, The TB control strategy in Ethiopia should be tailored to address MDR and latent infection.

Key words; Tuberculosis, prison, population structure, drug resistance, Jimma university

Contribution of this work to the research area:

This paper is the first paper to describe the population structure and drug resistance profile of *M. tuberculosis* in Ethiopia prisons which was unknown before. The observed low clustering rate combined with the strong association of TB with previous contact with TB patient at home and the observed shared cluster between prison isolate and community isolates clearly indicated the area of focus to control TB in Ethiopia.

1. Introduction

1.1. Tuberculosis in human history

Tuberculosis (TB) is used to be known by different names such as consumption, white plague, scrofula, King's evil, lupus vulgaris, and phthisis (Hart et al., 1996). These names were given as an attempt to describe the disease condition or indicate how the disease was dreadful in the past (Ducati et al., 2006). Even now in 21st century, tuberculosis is being continued to challenge the modern world and remained as one of the two top killer infectious diseases globally (Global tuberculosis report, 2015).

Tuberculosis is an infectious disease caused by bacilli which belongs to the genus mycobacterium; specifically *M.tuberculosis complex*. According to recent estimations mycobacterium genera was originated more than 150 million years ago (Daniel, 2006; Hayman, 1984). There is growing evidence that indicates the existence of an early progenitor of *M. tuberculosis* in East Africa as early as 3 million years (Comas et al., 2015; Gutierrez et al., 2005).

Tuberculosis has a long history. It is one of the oldest diseases existed before the begging of ancient literatures (Daniel, 2006). It has left its prints on human creativity and art (Hart et al., 1996). However, more concrete evidence was observed from Egyptian 5400 years old Mummies. In this human archeological finding Pott's deformities were reported (Crube et al., 1998). Written evidence about TB was documented in ancient literatures from Andean (South America), China and Egypt as early as 4,000 years ago (Fusegaw et al., 2003; Zink et al., 2003; Baronn et al., 1996).

Scientific concern for tuberculosis disease was actually started when Fracastero (1483-1553) inferred phthisis as an infectious disease (Hart et al., 1996). Later, Franciscus Sylvius (1614-

1672) described the characteristics nodules from autopsy and he named it as tubercles (Hart et al., 1996). During the golden era of microbiology Robert Koch has identified *Mycobacterium tuberculosis* in 1882 and demonstrated that TB is transmissible disease (Lakhtakia, 2014; Kaufmann, 2003). TB bacilli primarily causes pulmonary tuberculosis *which comprises* more than four fifth of TB cases seen in the world (Global tuberculosis report, 2015). MTBC can also disseminate to any part of the body and causes extra pulmonary TB (Ravigloin et al., 2012). The most common ones are pleural, lymph node, bones and joints, CNS and miliary tuberculosis (Herath et al., 2014; Ravigloin et al., 2012).

1.2. Classification of *M. tuberculosis or the M. tuberculosis complex*

Tuberculosis is caused by a number of related groups of bacteria collectively known as *Mycobacterium tuberculosis complex* (MTBC) (Forrellad et al., 2013). The complex includes a total of six to seven species grouped under the genus mycobacterium (*M .tuberculosis, M. bovis, M. africanum, M. Microtti, M. pinnipedii, M. cannetti and M. caprae*) (Forrellad et al., 2013). These species are genetically similar to each other sharing 99.9% DNA sequence identity in their chromosome but differs in primary host range and geographical distribution (Brites and Gagneux, 2015; Forrellad et al., 2013; Asiimwe et al., 2008).

1.3. Genotyping of *M.tuberculosis* complex

The discovery of molecular typing techniques has revolutionized the field of mycobacteriology and enabled to study of tuberculosis effectively. It has contributed significantly for the control and prevention of the disease. In the last decades, a large number of different molecular methods have been developed. These molecular methods discriminate the pathogen at the genus, species, and subspecies level (Ali et al., 2014).

Molecular strain typing (genotyping) have been further used to understand TB global distribution (Middlekoop et al., 2015). Genotyping of MTBC is becoming a vital tool in TB epidemiology through provision of vital information in relation to transmission dynamics , differentiating reactivation versus exogenous re infection, outbreak investigation, and mapping of the geographical spread of successful clones, including multi-drug-resistant strains (Middlekoop et al., 2015;Ali et al., 2014; Gupta et al.,2014;Tessema et al., 13).

In population based studies isolates that share the same genotype are considered as clusters and assumed epidemiologically linked (Barletta et al., 2015; Kato-Maeda et al., 2011). On the other hand, isolates of unique genotypes not shared by any other isolates within the population are considered as epidemiologically unrelated and resulted from reactivation of latent remote infection (Barletta et al., 2015; Kato-Maeda et al., 2011). This is only true by the assumption

that most of the peoples infected with MTBC will develop active form of the disease within the first 2-5 years (Barletta et al., 2015; Kato-Maeda et al., 2011).

So far several robust methods have been developed to depict the global population structure of tuberculosis. In line with this, Restriction-fragment length polymorphism (RFLP) analysis (Van Embden et al., 1993), Spoligotyping (Kamerbeek et al., 1997) and Mycobacterial Interspersed Repetitive Unit-Variable Number Tandem Repeats (MIRU-VNTRs) typing (Supply et al,. 2006; Kamerbeek et al., 1997) targeting different genes (DNA markers) to identify genotypes precisely were well described (Kato-Maeda et al., 2011). Accordingly, the global population structure of *M. tuberculosis* is defined in to seven phylogeographical lineages and 41 sub lineages (Figure 1) (Brites et al., 2015;Barletta et al., 2015; Yang et al., 2015;Hill et al., 2012).

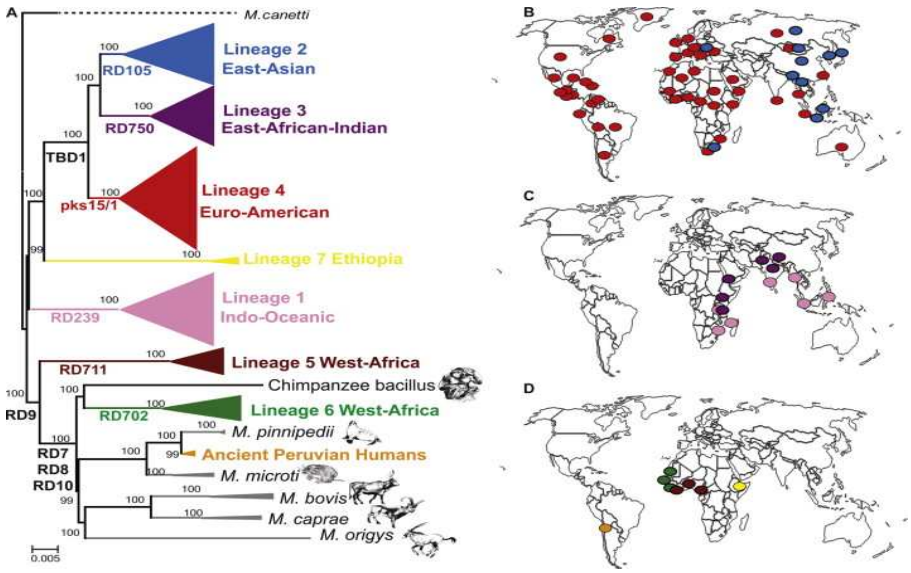

Figure 1. Pictorial representation of phylogenic diversity of the seven *M.tuberculosis complex* lineages with their global geographical distribution; lineage 2, 3 and 4 are modern lineages with TbD1 deletion Adopted from (Coscolla and Gagneux, 2014)

In our study, the isolated *M.tuberculosis* strains were analyzed at Research Center Borstel by using MIRU-VNTR typing customized kits (Genoscreen, Lilli, France). Basic strain classification. MLVA MTBC 15-9 nomenclature assignment was also done using the MIRU-VNTR*plus* database.

1.4. Pathogenecity of tuberculosis

Mycobacterium tuberculosis is transmitted by small airborne droplets generated from patients with pulmonary or laryngeal tuberculosis whenever they cough, sneeze, talk, or sing (Raviglion

et al., 2012). The diameter of the droplets generated should be small enough in size (1-5 μm) to reach deep into the lower respiratory tract as well as to remain suspended in the air (Riley et al., 1995; Wells, 1955). It is estimated that there might be as many as 3000 droplet nuclei released during a single cough (Maher et al., 1998). Droplets of larger size are efficiently excluded from the lower respiratory tract by the physical barriers of the nasopharynx and upper respiratory tract (Diamond et al., 1991).

Mycobacterium tuberculosis has evolved to cause infection in many, yet causing disease in few. This is demonstrated by the observed high TB infection rate with relatively low active TB disease prevalence seen globally. According to the WHO estimation 1/3 of the world's population harbors the bacilli. Nevertheless, only 2-23% of these are expected to develop disease during their life time (Ling and Flynn, 2015; Barry et al., 2009: Parrish et al., 1998). The probability of *M. tuberculosis* transmission is also associated with the duration and intensity of exposure, conditions of the shared environment, virulence feature of the infecting *M.tuberculosis* strains and TB index case related factors like sputum smear positivity (Dheda et al., 2010).

It is estimated that TB patients with single pulmonary cavity can produce as many as 10^5–10^7 bacteria per milliliter of sputum which resulted in smear positivity and infectiousness (Lau et al., 2016; Raviglion et al., 2012). The World Health Organization (WHO) also described sputum smear-positive pulmonary tuberculosis patients as the main source of TB infection in the community (http://www.who.int/mediacentre/factsheets/fs104/en/index.html). Furthermore, different literatures had documented the contribution of smear positive patients in TB transmission (Lau et al., 2016; Pagaoa et al., 2015; Marks et al., 2000).

2. Treatment and drug resistance

Tuberculosis is a curable disease. Combination of four drugs isoniazid (H), rifampicin (R), pyrazinamide (Z) and ethambutol (E) has been remained as the first-line treatment since 1994 (Bass et al., 1994; Treatment of tuberculosis, 2009 http://apps.who.int/iris/bitstream/10665/44165/1/9789241547833_eng.pdf?ua=1&ua=1). These anti TB drugs have been part of WHO-recommended tuberculosis treatment regimens since 1980s (Treatment of tuberculosis, 2009 http://apps.who.int/iris/bitstream/10665/44165/1/9789241547833_eng.pdf?ua=1&ua=1). The standard care of treatment for drug sensitive tuberculosis includes a combination of four drugs (RHZE) for at least 6 months (Bass et al., 1994). However, *M.tuberculosis* strains which are resistant to first line anti TB drugs were emerged since 1990 (Fischl et al., 1992). The

therapeutic options for patients infected with MDR-TB are very limited, less effective and more toxic than first-line anti TB drugs (Caminero et al., 2010).

3. Epidemiology of tuberculosis

3.1. Global Epidemiology

In the past decade the rate of new TB cases had been falling by 2% worldwide (Figure 2) (Global tuberculosis report, 2013). It is clear that stop TB target of halving TB prevalence in 2015 as compared to 1990 was not achieved globally (Global tuberculosis report 2015). The American and western pacific regions already achieved this target a head of time. The South East Asia and Europe have achieved the target recently (Global tuberculosis report 2015; Global tuberculosis report 2013). However, in African regions, the 2% reduction which was seen in the past decade was not good enough to achieve the Stop TB target though there is variability in performance among countries (Global tuberculosis report, 2015). For instance, Uganda, Tanzania and Ethiopia have achieved all the three global targets whereas; South Africa, Kenya and Zimbabwe have achieved the incidence reduction only. To the contrary, in countries like Mozambique, Democratic Congo and Nigeria the reduction was not good enough to achieve any of the three global TB targets set for 2015 (Global tuberculosis report, 2015).

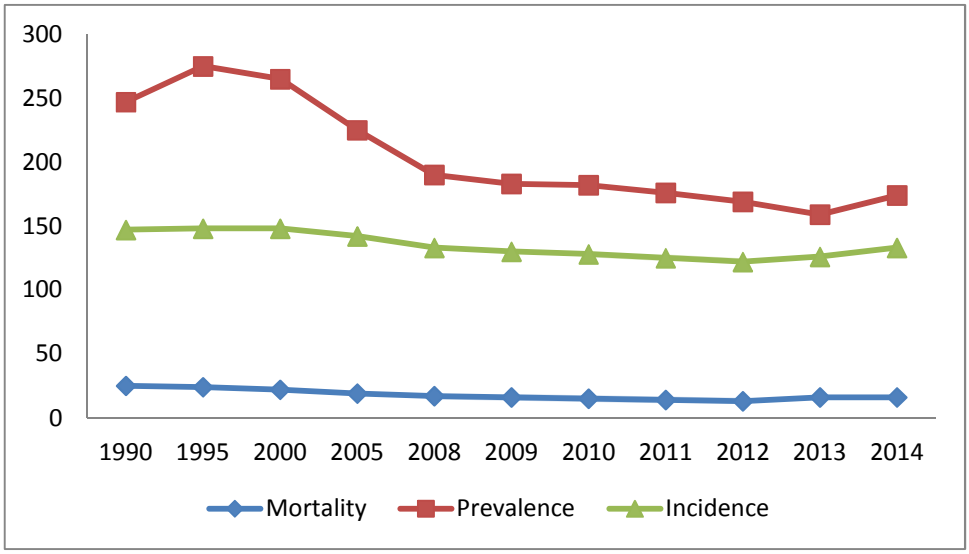

Figure 2 line graph of a 24 years Global tuberculosis incidence, prevalence and mortality trends per 100000 (Global tuberculosis reports, 1990- 2015)

3.2. Epidemiology of TB in Ethiopia

Tuberculosis is the third cause of death in Ethiopia next to pneumonia and HIV (Federal ministry of health, Health and health related indicators, 2014

http://www.cnhde.org.et/?page_id=19). Ethiopia has been ranked as fourth from African and tenth from the world in relation to the number of TB cases occurred annually (Global tuberculosis report, 2015). The country is also one of the 27 MDR high burden countries in the world (Global tuberculosis report, 2015). Ethiopian national prevalence survey conducted in 2010/2011 estimated the prevalence of TB as 277 per 100,000 (Kebede et al., 2014).

Ever since the year 1990 the government of Ethiopia mobilized all of its efforts to fight TB through early detection and treatment strategy. There was a massive utilization of health extension program throughout the country in the past 20 years. As a result, significant success was recorded in achieving the target set by WHO to stop TB (Figure 3) (Global tuberculosis report, 2015).Currently, Ethiopia has adopted a new, more precise end TB strategy from WHO. This new strategy targeted to reduce the incidence of TB as low as 10/100,000 population by the year 2035 (World Health Organization, The end TB strategy, 2015. http://www.who.int/tb/End_TB_brochure.pdf)

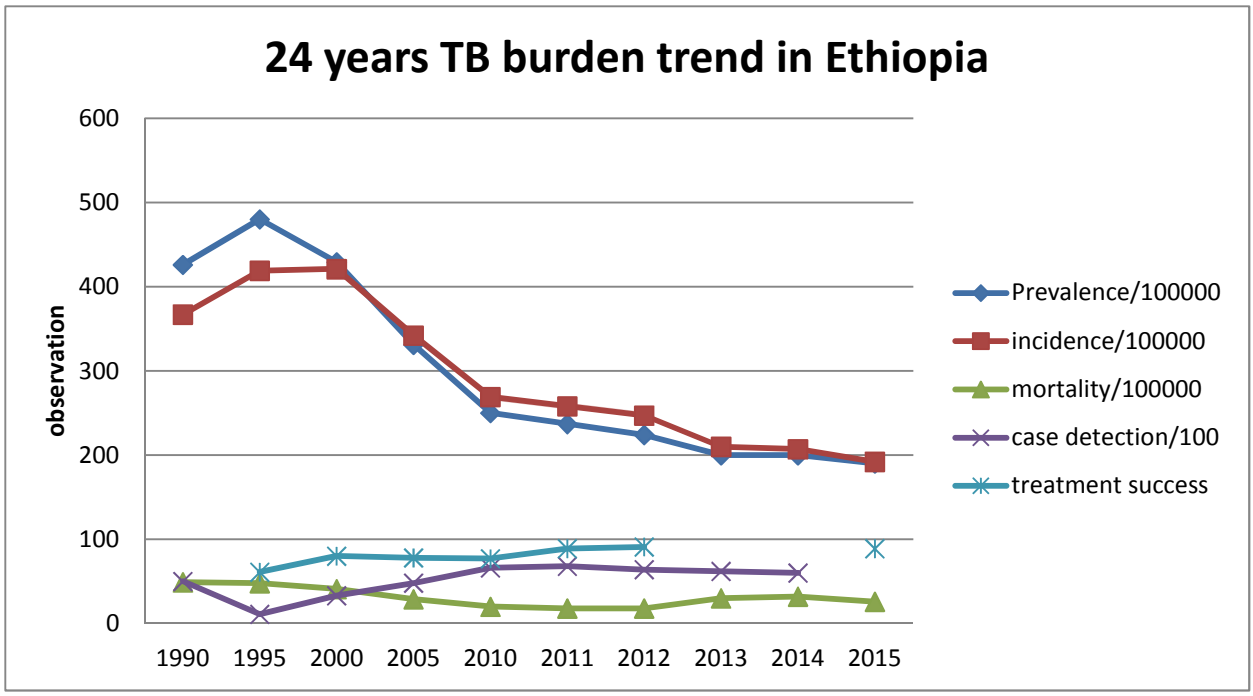

Figure 3; Line graph of a 24 years trend of tuberculosis in Ethiopia (WHO report, 1990-2015)

4. Factors that determine tuberclosis disease

Tuberculosis is a complex disease that involves different interlinked factors. In most of the cases these factors works synergestically and the occurance of one factor possibly predisposes to the other. For the sake of simplicity in this dissertation we have categorised factors that

influence TB from transmission to disease into three categories. These are pathogen factor, host factor and environmental factors.

4.1. Pathogen factor

The pathogenecity of a micro organisms are usually associated with the presence of virulence factor (Kędzierska and Hayes, 2016). *M.tuberculosis* is one of highly evolved bacteria that learned to live inside its host by overcoming the challenges. The irony thing is that, there is no classical bacterial virulence factors such as toxin and pathogenecity island identified from *M.tuberculosis* so far(Forrelland et al., 2013). However, Region of difference one (RD1) (Volkman et al., 2004: Guinn et al., 2004), Protein kinase G (Tiwari et al., 2009), Lipoarabinomannan (LAM) (Fukuda et al., 2013), the cord factor (trehalose 6,6′ -dimycolate), (Harland et al., 2008) were described to be associated with pathogenecity of *M.tuberculosis*. Apart from these some lineages of *M.tuberculosis* are more virulence than the others. For instance Beijing lineage was described to be associated with MDR tuberculosis epidemics. Strains of this lineage have been proposed to possess selective advantages in comparison to strains from other MTBC lineagesattributed to an increased capacity to acquire drug resistance, linked to hypermutability(Ford et al., 2013) .

4.2. Host factor including behavioral and social aspects

Susceptible host is one of the three epidemiology triads that determine the occurrence of disease. According to published researches on tuberculosis several host factors has been mentioned to determine the occurrence of TB disease. To mention some; genetic polymorphisms in TLR and HLA genes that affect host pathogen interaction or the strength of innate immune response (Fol et al., 2015), physiological factors like age and sex, nutritional factors, co-morbid and immune suppressive conditions such as diabetes and HIV (Hailu et al., 2014; Amare et al., 20013;Jeon et al., 2008), and behavioral factors such as smoking and alcohol abuse were well described in different studies (Ephrem et al., 2015;Wassie et al., 2014; Alavi-Naini et al., 2012).

4.3. Environment and other factors

Environmental factor which also includes the living condition and socio economic status is the other important factor for tuberculosis disease occurrence (Lacerda et al., 2014). TB is now becoming more of the problem of the poor (Marais et al., 2009). Most of the global tuberculosis burden is reported from low and middle income countries specifically from Africa and South East Asia (Global tuberculosis report, 2015). This part of the world is known by limited access for quality health service, extreme poverty, overcrowded living conditions and malnutrition (Marais et al., 2009).

Overcrowding is one of the most important factors to facilitate TB transmission (Marais et al., 2009). The number of peoples living per single room, size of the room, the ventilation condition of the room and the length of time spent in the room are different aspects of overcrowding. There are also facilities which are naturally prompted overcrowding. Some of these are day care centers, orphanages, military camps, universities, refugee camps and prisons. Unless the design of the rooms, the size of the rooms and the number of person per room are well managed it is highly likely for such facilities to favor TB transmission especially in low and middle income countries.

Prisons are one of the known overcrowded facilities which recently receiving attention globally. The global estimated number of people detained on any given day, is over 9 million (Dara et al., 2009). In countries like Ethiopia, the buildings and health infrastructure available in prisons is very deprived. As a result the problem of infectious diseases including tuberculosis could be high in prison facility.

5. Rationale

Tuberculosis (TB) is a major health problem in prisons and its prevalence was reported to be multiple times higher compared to that of the general population. Conditions such as overcrowding, malnutrition and limited access to medical care which often exist in prisons increase the risk of reactivation, transmission and poor prognosis of tuberculosis disease among inmates (Baussano et al., 2010; Habeenzu et al., 2007)

Several cross-sectional studies estimated the prevalence of TB in African prisons. Studies published from Cameroon, Zambia and Malawi reported relatively differing prevalence which was between 2.6 and 10 times higher than in the general population of the respective country (Banda et al., 2009;Habeenzu et al., 2007;Noeske et al., 2006). From Ethiopia, so far three studies were reported from 5 different prisons in Eastern Ethiopia, North Gondar zone and Gamo Gofa zone. The reported numbers for TB prevalence, 1,913, 1,482.3 and 629 TB cases per 100,000 inmates respectively, were comparable to that from other African settings (Zerdo et al., 2014;Moges et al., 2012;Abebe et al., 2011)

Prisons are regulated but not closed systems, due to the numbers of people who constantly enter, leave and re-enter into them. Therefore, prison health is a critical part of public health as health problems within and outside prisons are interrelated (Aerts et al., 2006). Every successful TB control program also requires effective TB control in prisons and failure to control TB in prisons has the potential to disrupt community TB control programs (O'Grady et al., 2011). Ethiopia is one of the 22 tuberculosis high burden countries with a recent TB prevalence

of 211 per 100,000 populations (Global tuberculosis report, 2014). According to the Ethiopian Human Right commission 2012 report, a total of 86,610 inmates were incarcerated in 119 prisons in Ethiopia (The ethiopian Human right comission, 2012 http://www.ehrc.org.et/LinkClick.aspx?fileticket=1uE7TO6QzbQ%3D&tabid=117).

Despite this fact, relatively little attention has been given to assess the condition of TB in Ethiopian prisons in the past. Those 3 studies mentioned above (Zerdo et al., 2014;Moges et al., 2012;Abebe et al., 2011) were relatively small and limited to 3 areas of the country. Further more, to date, no molecular epidemiological studies have been carried out to decipher MTBC population structure and drug susceptibility patter in prisons in Ethiopia.

6. Research questions and Objectives

6.1. Research questions

After understanding these existed knowledge gaps, the following research questions were asked. What is the magnitude of TB in prison? Does this magnitude is higher or lower than the reported population TB prevalence? What are the determinants of tuberculosis in prison? Is the population structure of MTBC isolates from prison differs from isolates from the community? What does the drug susceptibility pattern of MTBC isolates looks like? To answer these questions, this research was planned as part of my PhD aiming to contribute something for prison TB control effort of Ethiopia.

6.2. Objective

6.2.1. General objective

To assess the magnitude of tuberculosis, risk factors, drug resistance patterns and MTBC population structure in Ethiopian prisons.

6.2.2. Specific Objective

- ✓ To determine the prevalence of *tuberculosis* in Ethiopian prisons
- ✓ To identify possible risk factors for tuberculosis in prison.
- ✓ To identify dominant strains/genotypes of *M. tuberculosis* in prisons
- ✓ To determine first line drug susceptibility patterns of isolated *M. tuberculosis* genotypes
- ✓ To determine the association between drug susceptibility and strain of *M. tuberculosis*

7. Methodology

7.1. Study setting

Ethiopia is administratively organized within nine regions and two federal cities. Among these, Oromia, Amhara and Southern Nations, Nationalities and Peoples Regional State (SNNPRS) are the three biggest regions with a total population of approximately 67,730,002 (more than 80% of total Ethiopian population). Harari is the smallest regional state with a population of only 210,000 (Central statistics Agency, 2012). Due to logistic reasons such as cooperation with investigators and accessibility from the study centre at Jimma University the participating prisons for this study were selected from Oromia, SNNPRS and Harari regional states (Please see publication A for detail information).

7.2. Sample size estimation

A total of 13 zonal administrative level prisons were randomly selected by lottery method. Accordingly, seven out of 17 and five out of 13 prisons were drawn from Oromia and SNNPRS, respectively, while the only prison of Harari regional state was included in the study. By this approach, ca. 35% of the total prison population of the included regional states was represented in this study. For molecular epidemiology study all isolated bacteria from prison study and 100 strains from respective hospitals were included (Please see the methodologies of publication A and B attached in this document).

7.3. Data and sample collection

A cross sectional study was done from January 2013 to June 2015. Study activities were conducted in one prison after the other (Please see the methodology of publication A and B for detail explanation).

7.4. Statistical analysis

All data were recorded on standardized data collection forms. Data were double-entered in an excel data base. Analysis was done by STATA version 10. In the descriptive analysis, for categorical variables proportions with 95% confidence level and for continuous variables means with 95%CI were calculated. Univariable logistic regression analysis was conducted to analyze the association between individual risk factors TB-diagnosis/clustering; a p-value of <0.05 was considered as significant. Factors with significant association were used to build the final multivariable logistic regression model using a forward elimination approach. Likelihood ratio test was performed to confirm significant association of each risk factor with the outcome in multivariable regression model and to test for a linear trend for categorical variables. Almost all of the statistical analysis were done by the PhD candidate. Specifically,data entry, cleaning, descriptive analysis, Univariate analysis and Multivariate analysis by using STATA version 10

software. Furthermore, advanced logistic regression and molecular typing data was analyzed with the help of direct LMU supervisor and Borstel collaborators.

8. Publications

8.1. Brief summary of first publication

The first paper was published in December 2015. The paper has mainly addressed the magnitude and risk factors of tuberculosis in Ethiopian prison. A total of 15 495 inmates incarcerated in 13 zonal prisons were screened and 765 participants who fulfilled the inclusion criteria were included. The study was a cross sectional study done from January 2013 to December 2013 on inmates incarcerated in 3 regional states. The prevalence and risk factors were determined and clearly presented in the result section of the attached paper. One interesting finding was the linear relation between TB prevalence in prison and the location of the prison from the central Ethiopia Addis Ababa which indicated the need to address TB in remote part of the country. All the major findings were also discussed and interpreted in comparison with other previous study reports done in Ethiopia or elsewhere to give broader horizon for the reader. Finally conclusion and recommendations were forwarded for stake holders working in TB prison in Ethiopia in particular for better control of the disease and global scientific communities working in tuberculosis in general. For detail information please see the attached full text in this paper or visit Ali et al., 2014 from plos one (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4671540/pdf/pone.0144040.pdf)

PLOS | ONE

RESEARCH ARTICLE

Prevalence of Pulmonary Tuberculosis among Prison Inmates in Ethiopia, a Cross-Sectional Study

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Competing Interests: The authors declare that this manuscript was approved by all authors in its form and that no competing interest exists.

Abstract

Setting

Tuberculosis (TB) is one of the major health problems in prisons.

Objective

This study was done to assess the prevalence and determinants of active tuberculosis in Ethiopian prisons.

Design

A cross-sectional study was conducted from January 2013 to December 2013 in 13 zonal prisons. All incarcerated inmates underwent TB symptom screening according to WHO criteria. From identified TB-suspects two sputum samples were analyzed using smear microscopy and solid culture. A standardized questionnaire assessing TB risk factors was completed for each TB suspect.

Results

765 (4.9%) TB suspects were identified among 15,495 inmates. 51 suspects were already on anti-TB treatment (6.67%) and 20 (2.8%) new culture-confirmed TB cases were identified in the study, resulting in an overall TB prevalence of 458.1/100,000 (95%CI: 350-560/100,000). Risk factors for active TB were alcohol consumption, contact with a TB case before incarceration and no window in prison cell. HIV prevalence was not different between TB suspects and active TB cases. Further, the TB burden in prisons increased with advancing distance from the capital Addis Ababa.

Conclusions

The overall TB prevalence in Ethiopian prisons was high and extremely variable among different prisons. TB risk factors related to conditions of prison facilities and the impact of

implemented TB control measures need to be further studied in order to improve TB control among inmates.

Introduction

Tuberculosis (TB) is a major health problem in prisons and its prevalence was reported to be multiple times higher compared to that of the general population. Conditions such as overcrowding, malnutrition and limited access to medical care which often exist in prisons increase the risk of reactivation, transmission and poor prognosis of tuberculosis disease among inmates [1,2].

Several cross-sectional studies estimated the prevalence of TB in African prisons. Studies published from Cameroon, Zambia and Malawi reported relatively differing prevalence which was between 2.6 and 10 times higher than in the general population of the respective country [1,3,4]. From Ethiopia, so far three studies were reported from 5 different prisons in Eastern Ethiopia, North Gondar zone and Gamo Gofa zone. The reported numbers for TB prevalence, 1,913, 1,482.3 and 629 TB cases per 100.000 inmates respectively, were comparable to that from other African settings [5–7].

Various factors were specified as determinants of tuberculosis in prisons. Among them low socio economic status, history of anti TB treatment before incarceration, previous contact with TB patients, low body mass index and HIV infection were frequently associated with active TB in prisons in different studies [1,5,6,8,9].

Prisons are regulated but not closed systems, due to the numbers of people who constantly enter, leave and re-enter into them. Therefore, prison health is a critical part of public health as health problems within and outside prisons are interrelated [10]. Every successful TB control program also requires effective TB control in prisons and failure to control TB in prisons has the potential to disrupt community TB control programs [11]. Ethiopia is one of the 22 tuberculosis high burden countries with a recent TB prevalence of 211 per 100,000 populations [12]. According to the Ethiopian Human Right commission 2012 report, a total of 86,610 inmates were incarcerated in 119 prisons in Ethiopia [13].

Despite this fact, relatively little attention has been given to assess the condition of TB in Ethiopian prisons in the past. Those 3 studies mentioned above [5–7] were relatively small and limited to 3 areas of the country. To the best of the author's knowledge, this is the first huge study conducted in more than 15,000 inmates of 13 prisons in three different regional states of Ethiopia to systematically determine the prevalence of TB and its risk factors in Ethiopian prisons.

Materials and Methods

Study setting

Ethiopia is administratively organized within nine regions and two federal cities. Among these, Oromia, Amhara and Southern Nations, Nationalities and Peoples Regional State (SNNPRS) are the three biggest regions with a total population of approximately 67,730,002 (more than 80% of total Ethiopian population). Harari is the smallest regional state with a population of only 210,000 [14]. Due to logistic reasons such as cooperation with investigators and accessibility from the study centre at Jimma University the participating prisons for this study were selected from Oromia, SNNPRS and Harari regional states. These three regional states together cover an area where almost 60% of the total Ethiopian population resides [14]. Oromia regional

state had 37 (17 zonal and 20 district) prisons, while SNNPRS had 23 (13 zonal and 10 district) prisons. Harari regional state had only one zonal prison [13]. Zonal administrative prisons are the largest prisons in Ethiopian context. A total of 13 zonal administrative level prisons were randomly selected by lottery method. Accordingly, seven out of 17 and five out of 13 prisons were drawn from Oromia and SNNPRS, respectively, while the only prison of Harari regional state was included in the study. By this approach, ca. 35% of the total prison population of the included regional states was represented in this study. All zonal prisons had a small clinic which was equipped to handle emergency situations and to treat frequent infections with antibiotics. For (microbiological) diagnosis and treatment of more complicated, severe and chronic diseases, including TB, inmates were referred to nearby hospitals or health facilities. In diagnosed TB patients treatment was provided and supervised by prison clinics according to national guidelines.

Data and sample collection

A cross sectional study was done from January 2013 to December 2013. Study activities were conducted in one prison after the other in the following order: Jimma, Nekemte, Ambo, Wolkite, Shashemene, Asella, Bonga, Mizan, Yabelo, Dilla, Sodo, Asebeteferi/Chiro, Harar prison. Prison inmate health committee members together with health professionals working in prison were trained on scientific purposes, ethical aspects and data collection procedures of this study.

In a first step, all inmates were registered with support of the health committee members in each prison. Then TB-symptom screening was conducted by the research staffs using a questionnaire provided by WHO [15]. All inmates who were 18 years or older and who fulfilled at least one of three screening criteria listed below were considered as TB suspects and included in the study [15].

1. Inmates with a score of 5 according to WHO recommended tuberculosis suspect identification criteria: Cough of two weeks duration (scored as 0 or 2), sputum production (scored as 0 or 2), chest pain (scored as 0 or 1), recent loss of appetite (scored as 0 or 1) and loss of weight in last 3 months (scored as 0 or 1).
2. Inmates who had history of anti TB treatment in the past five years.
3. Inmates living with HIV.

Each study participant was examined and interviewed by a study clinician using a predesigned questionnaire (S1 File). All study participants were counseled and tested for HIV in line with the Ethiopian national algorism for HIV testing and counseling. Two early morning sputum samples were collected from each participant on two consecutive days, after instructions about technique of coughing and sputum quality were provided by study staff. The quality of sputum was checked upon reception, saliva and soil contaminated specimens were rejected and participants were asked to bring another specimen. The first early morning sputum was stored at 2–8°C for a maximum of one week until transported to Jimma University Mycobacteriology Laboratory where sputum culture was performed. The second early morning sputum was processed in prisons for immediate diagnosis of tuberculosis by smear-microscopy after Ziehl-Neelsen staining and was then re-read by an experienced microbiologist in Jimma. Completeness and accuracy of all study documents, including the questionnaire, screening logs and laboratory log books, were checked daily by the local principal investigator. Sputum received in Jimma University Mycobacteriology laboratory was processed using Specimen Digestion/Decontamination Kit following the manufacturer´s instructions [16]. The processed samples were inoculated on LJ slants (Lowenstein-Jensen Medium) as described by the

manufacturer [17], including growth control using H37RV strain in 5% of LJ-slopes and sterility control for all used slopes. Apart from manufacturer instructions all assays were performed according to the implemented standardized operating procedures.

Data analysis

All data were recorded on standardized data collection forms. Data were double-entered in an excel data base. Analysis was done by STATA version 10. In the descriptive analysis, for categorical variables proportions with 95%CI and for continuous variables means with 95%CI were calculated. Univariable logistic regression analysis was conducted to analyze the association between individual risk factors and TB-diagnosis; a p-value of <0.05 was considered as significant. Factors with significant association were used to build the final multivariable logistic regression model using a forward elimination approach. Likelihood ratio test was performed to confirm significant association of each risk factor with the outcome in multivariable regression model and to test for a linear trend for categorical variables.

Ethical considerations

Ethical clearance was obtained from Jimma University Ethical Review Board. Written consent was sought from each study participant. Directly observed treatment short course was started for newly diagnosed TB patients in collaboration with the nearby health center. Newly diagnosed HIV positive participants were linked to the nearby health institutions for follow up and possible initiation of anti-retroviral therapy. Permission to conduct the research was granted by relevant prison authorities.

Results

In this study, a total of 15,495 inmates incarcerated in 13 different prisons underwent TB symptom screening. Seven hundred sixty five (4.9%), (95%CI: 4.6%-5.2%) fulfilled the TB screening criteria out of whom 51 (6.7%) were already diagnosed earlier by Ziehl-Neelsen smear microscopy and placed on anti tuberculosis treatment during incarceration. Among the remaining 714 participants 20 (2.8%) were newly diagnosed with active pulmonary tuberculosis in this study. Ten (50%) of them were positive by smear microscopy. Thus, TB prevalence among suspects was 9.2% (71/765), (95%CI: 7.2–11.4), and among all prisoners it was 0.46% (71/15,495), (95%CI: 0.35–0.57) (Fig 1)

Characteristics of study participants

Out of the 765 participants, 96.8% were male (Table 1). The mean age was 32.5 (95%CI: 31.5–33.4). 4.44% (34/765) of participants were tested positive for HIV. Three of them were also diagnosed with active TB, resulting in a HIV-prevalence of 4.23% (3/71) among TB cases. The majority (68.5%) were farmers before incarceration. Approximately two thirds were following Muslim (38.6%) or Orthodox (30.7%) religions. Most participants (66.9%) were married. More than one third (39.1%) were either illiterate or had no formal education (Table 1). The mean duration of stay in prison was 26.1 months (95%CI: 24.1–28.1) at the time point when the study was conducted, with no difference between TB suspects and confirmed TB cases. Eighty eight percent of participating inmates had no history of incarceration before the current sentence.

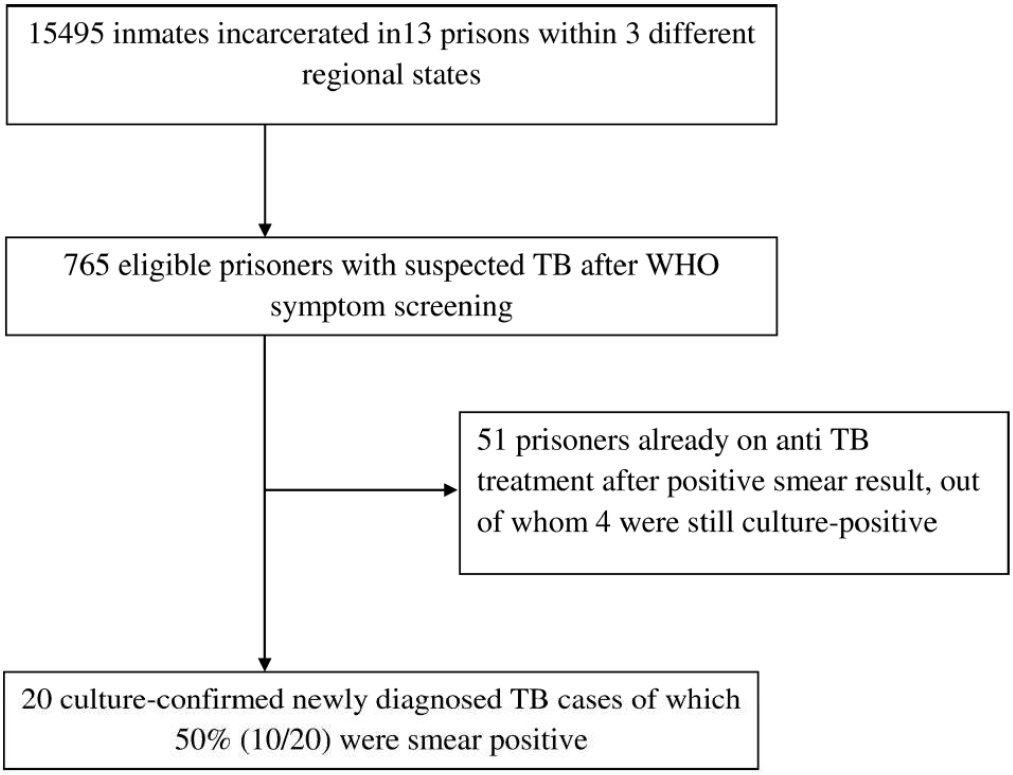

Fig 1. Overall study flow. Total prisons included, inmates screened, identified suspects and detected TB cases were presented by absolute number.

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Variability of TB-prevalence among different prisons

Considering the 51 already existing and the 20 newly diagnosed TB cases, the overall point prevalence of tuberculosis in these 13 prisons was 458.1 (95%CI:350–560) per 100,000 inmates, though there was great variability among prisons (Table 2). The highest TB prevalence was observed in Dilla prison (SNNPRS) with 1528 cases per 100,000 inmates. Opposed to that, there was no TB case detected at Wolkite (SNNPRS) and Asebeteferi/Chiro prisons (Oromia). The point prevalence of newly diagnosed TB was 129 (95%CI: 70–190) per 100,000 inmates with a variability that ranged from no new TB case detected in five prisons to 887.6 new TB cases per 100,000 which was observed at Yabelo prison (Oromia).

Among the different regional states the SNNPRS had the highest tuberculosis burden with an overall prevalence of 618.8 (95%CI: 420–820) per 100,000 inmates. The prevalence of TB in Harar and Oromia regional states were 529.5 (95%CI: 160–590) and 330.5 (95%CI: 210–460) per 100,000 inmates, respectively. We found a linear trend in prevalence of tuberculosis with advancing distance of the prisons from the centre of Ethiopia (Addis Ababa). Prisons within a radius of below 200km distance from Addis Ababa had the lowest TB prevalence of 97.98 (95% CI: 10–210) per 100,000 inmates while the highest TB prevalence of 804 (95% C.I: 580–1020) per 100,000 inmates was observed in prisons located more than 400km away from Addis Ababa (S1 Fig), (OR = 3.60, 95% CI: 2.24–5.70, $p<0.0001$).

Table 1. Socio demographic characteristics and TB risk factors of participants.

Characteristic		Total N = 765	Proportion in % (95%CI)
Sex			
	Male	741	96.86 (95.6–98.1)
Age			
	≤ 45 Years	651	85.1 (82.6–87.6)
Religion			
	Muslim	295	38.61 (35.2–42.1)
	Orthodox	235	30.76 (27.5–34.0)
	Protestant	200	26.18 (23.1–29.3)
	Ahizab	18	2.36 (1.3–3.4)
	Catholic	6	0.79 (0.2–1.4)
	Others	10	1.31 (0.5–2.1)
Occupation			
	Farmer	524	68.5 (65.2–71.8)
	Student	86	11.24 (9.0–13.5)
	Merchant	42	5.49 (3.9–7.1)
	Employee	30	3.92 (2.5–5.3)
	No job	10	1.31 (0.5–2.1)
	Driver	7	0.92 (0.2–1.5)
	Others	66	8.63 (6.6–10.6)
Marital Status			
	Married	512	66.93 (63.6–70.2)
	Single	229	29.93 (26.7–33.2)
	Divorced	18	2.35 (1.3–3.4)
	Widowed	6	0.78 (0.2–1.4)
Education			
	Illiterate	286	37.39 (33.9–40.8)
	Read & write	13	1.70 (0.8–2.6)
	1–4 grade	157	20.52 (17.7–23.4)
	5–8 grade	208	27.19 (24.0–30.3)
	9–12 grade	83	10.85 (8.6–13.1)
	>12 grade	18	2.35 (1.3–3.4)
History of incarceration			
	No	678	88.63 (86.4–90.1)
HIV serology			
	Negative	**731**	**95.56 (94.1–97.0)**

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In all prisons, the mean number of incarcerated inmates per cell was 134.8 (95%CI: 129.2–140.3). In prisons like Ambo, Asebeteferi/Chiro, Dilla and Mizan the number of inmates incarcerated per square meter area was greater than one. The lowest number of inmates incarcerated per square meter was seen in Wolkitie with around 0.5. However there was no significant association found between the number of inmates per square meter and TB prevalence in prisons (OR = 1.59, 95%CI: 0.97–2.59, p = 0.07).

Risk factors associated with active TB

In this study, 71 smear or culture confirmed TB cases were compared with 688 inmates without TB to identify risk factors for active TB disease. Alcohol consumption and history of contact

Table 2. Prison characteristics and prevalence of TB by prison.

Prison	Total inmates	Total area (m^2)	Inmates/m^2	Prison distance from Addis Ababa in km	TB suspects identified (%)	Prisoners already on TB treatment (%)*	Newly diagnosed TB cases (%)*	Smear positives among new cases (%)*	Prevalence newly diagnosed TB cases per 10^5	Prevalence all TB cases per 10^5
Ambo[1]	1602	1413	1.13	126	45 (2.8)	1 (2.2)	-	-	-	62.4
Asebeteferi[1]	1243	942	1.32	326	28 (2.3)	-	-	-	-	-
Asella[1]	1067	2270	0.47	175	53 (5.0)	1 (1.9)	1(1.9)	0 (0)	93.7	187.4
Bonga[2]	1306	nd	nd	465	58 (4.4)	14 (24.1)	1(2.3)	1(2.3)	76.6	1148.5
Dilla[2]	916	759.50	1.21	405	58 (6.3)	8 (13.8)	6 (12.0)	3 (50.0)	655.0	1528.4
Harar[3]	1511	1944.24	0.78	526	60 (4.0)	7 (11.7)	1(1.9)	0 (0)	66.2	529.5
Jimma[1]	1267	2133	0.59	355	140 (11.0)	4 (2.8)	-	-	-	315.7
Mizan[2]	1929	1505	1.28	561	59 (3.0)	2 (3.4)	2 (3.5)	1 (1.7)	103.7	207.4
Nekemte[1]	1172	nd	nd	328	70 (6.0)	7 (10.0)	-	-	-	595.7
Shashemene[1]	1139	1426	0.80	254	83 (7.3)	2 (2.4)	1(1.2)	0 (0)	87.8	263.4
Sodo[2]	1274	1294	0.98	383	38 (3.0)	1 (2.6)	2 (5.4)	2 (5.4)	157.0	235.5
Wolkite[2]	393	807.50	0.49	155	16 (4.1)	-	-	-	-	-
Yabelo[1]	676	819	0.83	570	57(8.4)	4 (7.0)	6 (11.3)	3 (5.6)	887.6	1479.3
Total N/Mean	**15495**	**15313.2**	**0.85**	**356.07**	**765 (4.9)**	**51 (6.67)**	**20 (2.8)**	**10 (1.4)**	**129.0**	**458.1**

%: percentage, m^2: square meters, km: kilometers, nd: no data, SNNRS: south nations and nationalities regional state

* among identified TB suspects

[1] Oromia Region

[2] SNNPRS

[3] Harare Region

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with TB patient at home were significantly associated with active TB disease, while the availability of a window in the prison cell reduced the probability of TB in prisoners (Table 3). In our study population, risk factors such as education level, mean duration of stay in prison,

Table 3. Logistic regression analysis of risk factors for active TB disease in prisoners.

Variable	Univariable analysis*		Multivariable analysis*	
	COR (95%CI)	p-value	AOR (95%CI)	p-value
Incarcerated in cell with window	0.25 (0.15–0.42)	<0.001	0.26 (0.16–0.45)	<0.001
Alcohol consumption	1.98 (1.20–3.21)	0.008	2.04 (1.20–3.46)	0.008
TB case contact at home	1.59 (1.18–2.15)	0.002	1.49 (1.08–2.06)	0.02
TB case contact in prison	1.15 (0.82–1.61)	0.41	-	-
Religion	1.09 (0.91–1.30)	0.37	-	-
Education	0.93 (0.79–1.09)	0.35	-	-
Duration of stay in prison	1.06 (0.86–1.32)	0.58	-	-
Cigarette smoking	0.93 (0.73–1.17)	0.53	-	-
Khat chewing	1.12 (0.84–1.51)	0.44	-	-
Positive HIV serology	0.9 (0.28–3.14)	0.91	-	-

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

*Six participants without active TB disease were removed from analysis due to incomplete data set

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cigarette smoking, chewing khat and positive HIV-status were not significantly associated with TB disease (Table 3).

Discussion

Recently three studies were published on the prevalence of tuberculosis in Ethiopian prisons. However, these studies included only five prisons in total which covered an average of 2.2%, 3.6% and 4.4% of total inmates incarcerated at that time in Ethiopia, respectively [5–7]. To the best of our knowledge, this is the first large scale study performed in 13 prisons located in the south western, southern and eastern part of the country which covered about 18% of inmates incarcerated in whole Ethiopia.

In this study the overall prevalence of TB was 458.1 per 100,000 inmates. In comparison to data published by WHO in 2014 the observed prevalence in our prison study was still more than two times higher than that estimated for the general Ethiopian population which was 211 per 100,000[12]. It is not a surprise to see such an increment of TB considering conditions such as overcrowding, which could also be observed in some prisons in this study, as well as nutritional factors and limited access to medical care existing in prisons, On the other hand, the observed overall TB prevalence in this study was much lower than reported from the previous Ethiopian studies [5–7], conducted in 2008 to 2012. Possible reasons for that might be differences in study size, a low sensitivity of the relatively strict inclusion criteria applied in our study and the low HIV-prevalence of 4.4%. The lower TB prevalence in our prison study might be also associated with the decline of TB prevalence in the general population observed for Ethiopia in the last five years. The national TB survey of 2010/2011 reported a TB prevalence of 277/100000 [18], while in 2013 the prevalence of TB in the general population was declined to 211/100000 [12]. Further, Ethiopia is among those African countries which had achieved the 2015 global targets announced by the Stop TB partnership, reflecting the efforts of the national government and its allies to control TB in the country, including TB in prisons.

Interestingly, we observed a great variability of TB prevalence among different prisons, varying from no TB case detected in two prisons while 1,528.4 TB cases per 100,000 prisoners found in a prison with the highest TB prevalence. The data suggest that there might be relevant differences in the efficiency and commitment of the prison health workers or responsible authorities to implement systematic and effective TB-control strategies. For instance, in some prisons facilities was no segregation area for newly diagnosed or infectious TB patient inmates. Further, inmates incarcerated in rooms without a window had a four times higher TB risk than those incarcerated in rooms where a window was present. Other prison studies from Ethiopia and Thailand which assessed the effect of ventilation through windows observed similar findings [5,8]. On the other hand, crowding measured as number of inmates per square meter of prison cell was not associated with TB diagnosis in this study. Of note, only 28.2% of all prevalent TB cases were newly diagnosed in this study. The remaining cases were already on TB-treatment after positive smear-microscopy result. This indicates that in some studied prisons the implemented strategies for TB-detection and treatment were effectively installed. Additionally, distance from the capital Addis Ababa might play a role in the occurrence of TB in prisons. This finding is interesting and cannot be explained by greater negligence of TB in more remote prisons only as the number of both, already treated cases and newly diagnosed cases, were equally increased in peripheral prisons. As inmates who reported a previous TB-contact at home or alcohol consumption had a significant higher risk to be diagnosed with TB one could speculate that the higher TB prevalence in remote prisons also relates to certain risk behavior and consequently a relatively higher TB burden in certain sub-groups of the general population in remote areas. A decreased awareness of tuberculosis and its transmission which might be

associated with illiteracy and lower socio-economic standard, a poorer health system infrastructure including the lack of well-trained health professionals and the geo-climatic conditions one can find in remote areas of southern Ethiopia might also explain the observed differences in TB-prevalence among the prisons in this study.

HIV-infection was not an independent risk factor for TB-infection in our study. However, this could be most likely explained by the comparably low HIV-prevalence in those prisons which were included in this study compared to other publications [9,19,20].

Although performed in a large number of prisoners our study has several shortcomings which demand that the results are interpreted with caution. First, the relatively strict inclusion criteria for symptomatic prisoners which demand productive cough for at least 2 weeks plus one additional TB symptom might have resulted in an underestimation of TB-prevalence. This assumption might be supported by the low proportion (4.9%) of TB suspects in this study compared to other publications [5,6]. On the other hand, in most settings the symptom-based WHO screening is the best tool available as extensive screening using microbiological tests or chest x-ray in all inmates are not affordable to many African countries. Further, the delay between sputum collection and processing for culture might have led to an increased number of samples without a positive result for *M.tb* due to growth of contaminating flora in 12.5% of cultures. Second, although the socio-demographic and risk factor questionnaire was translated into local languages and study staff was trained on its application bias due to over or under reporting of risk factors could still have occurred as prisoners might not remember facts correctly or did not want to reveal the true information. Third, our findings might not be representative for the prison population of the whole country due to restriction to prisons in only three regional states and the selection process of studied prisons. Given the high variability of TB prevalence and prison characteristics we observed among the included prisons one could speculate that there might be even a higher diversity among prisons across the whole country.

Conclusion

The average TB prevalence in prison inmates is twice higher than the prevalence in the general population and a great variability of prevalence among different prisons existed. This variability and the higher TB burden in prisons located far from the capital suggest that the national TB control measures are either not similarly implemented in the different prisons or have a differing impact on TB-control in specific prison environments or study populations. This needs further attention and future studies should focus on risk factors related to the individual but also on factors inherent to the general population and the prison environment including the functioning of TB control strategies. Ongoing attention to those prisoners with specific TB risk factors such as TB contact at home, alcohol drinking and, still, with known positive HIV-status is demanded.

Supporting Information

S1 File. Questionnaire used for data collection.
(PDF)

S1 Fig. TB prevalence in prisons by distance from Addis Ababa. <200km from Addis Ababa: Ambo, Wolkite and Asella prisons, 200-400km from Addis Ababa: Shashemene, Nekemte, Sodo, Asebe Teferi/Chiro, Jimma´prisons, >400km from Addis Ababa: Bonga, Mizan, Dilla, Yabelo and Harar prison.
(TIF)

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Author Contributions

Conceived and designed the experiments: SA AR AH TL MH MP NH AW. Performed the experiments: SA AR AH AW. Analyzed the data: SA AR AH. Contributed reagents/materials/analysis tools: SA AR AH TL MH MP AW NH. Wrote the paper: SA AR AH TL MH MP AW NH.

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8.2. Brief summary of second publication

In this paper, MTBC population structure, transmission dynamics and drug susceptibility pattern of isolates from prisons and communities of southern, southwest and eastern part of Ethiopia were reported using combined spoligotyping and MIRU-VNTR typing methods. A total of 127 strains collected from Oroimia, SNNRS, Somali, Dire Dawa and Harari regional states. These strains were transported to German, Borstel for molecular finger printing and DST.

Diverse population structure of MTBC with no difference between the prison and community isolates were observed as indicated on radial UPGMA tree based on MIRU-VNTR analysis. Ethiopian_H37Rv like genotype was the dominant strain seen in both prison and community isolates. Isolates from some regional states showed higher clustering rate than others. For instance, isolates from Somali regional state are more clustered than others. This finding is very important finding due to the fact that TB is still actively transmitted more in this regional state. The drug susceptibility patterns of the isolates were the same among prison and community isolates.

A total of 24.77% of the isolates were resistance against at least one first line anti TB drugs. Furthermore, the MDR rate was found out to be 2.47% on strains isolated from community. Drug resistance was equally distributed among different genotypes isolated from prison or communities. The finding was discussed and interpreted in detail in line with available literatures and current knowledge regarding population structure and DST of MTB.

Finally conclusions and recommendations were forwarded to indicate the future direction of TB control or research in Ethiopia. Please read the attached full text article or visit https://bmcinfectdis.biomedcentral.com/articles/10.1186/s12879-016-2041-x for further detail information.

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Drug resistance and population structure of *M.tuberculosis* isolates from prisons and communities in Ethiopia

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Abstract

Background: The population structure and drug resistance pattern of *Mycobacterium tuberculosis* complex (MTBC) isolates in Ethiopian prisons and some communities is still unknown.

Methods: A comparative cross sectional study was conducted on 126 MTBC strains isolated from prisons and communities in southwestern, southern and eastern Ethiopia. Phenotypic drug susceptibility testing was performed with the MGIT960 system. Combined 24-loci *Mycobacterium* interspersed repetitive unit-variable number tandem repeat and spacer oligonucleotide typing methods were used to study the MTBC population structure. The obtained data from prisons and communities were compared using statistical tests and regression analysis.

Results: A diverse population structure with 11 different lineages and sub-lineages was identified. The predominant strains were the recently described Ethiopia_H37Rv like (27.52%) and Ethiopia_3 (16.51%) with equal lineage distribution between prisons and communities. 28.57% of prison strains and 31.82% of community strains shared the identical genotype with at least one other strain. The multidrug-resistance (MDR) prevalence of the community was 2.27% whereas that of prisons was 9.52%. The highest mono resistance was seen against streptomycin (15.89%).

Conclusion: Tuberculosis in communities and prisons is caused by a variety of MTBC lineages with predominance of local Ethiopian lineages. The increasing prevalence of MDR MTBC strains is alarming. These findings suggest the need for new approaches for control of MDR tuberculosis in Ethiopia.

Keywords: TB genotypes, Drug resistance, TB in Ethiopia

Background

Despite recent achievements seen in the fight against tuberculosis (TB), it still remains a significant cause of morbidity and mortality in Ethiopia [1]. This situation is worsened by an increase in prevalence of multidrug-resistant (MDR) *Mycobacterium tuberculosis* complex (MTBC) strains, defined as resistance to at least the two most powerful first line anti-tuberculosis drugs; isoniazid (INH) and rifampicin (RIF) [2].

The recent TB drug resistance survey, conducted in Ethiopia from 2011 to 2013, revealed an increase in MDR-TB with a prevalence of 2.3% and 17.8% among new and previously treated cases, respectively [3]. These data indicate that MDR-TB has been becoming a significant public health threat in the country. The occurrence and transmission of MDR-TB in confined environment like correctional facilities could further worsen the problem [4]. Due to the specific conditions found in prisons such as crowded living conditions, a large number of inmates could be infected and develop active TB disease within a short period of time. Accordingly, TB incidence and MDR rates in prisons have been found to be higher compared to that reported in the general population in several studies [5–7]. This is likely to have an impact

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also on the civilian population e.g., by direct transmission via visits, prison staff or later spread in community after release from prison [7, 8]. Importantly, latently infected inmates may become a reservoir of MDR-TB and a threat to the communities. In some studies performed to identify the risk factors for TB in communities previous incarceration history was described as a risk factor [9, 10].

While a number of studies confirm that TB rates are higher in prisons [7, 8, 11] little direct evidence about strain diversity and population structure of *M.tuberculosis* within prisons and the interaction of the prison setting with the community e.g., by molecular epidemiological studies is available [4, 12].

Increasing evidence suggests that the underlying genetic diversity of the MTBC has a significant impact on the pathogenicity and immunogenicity of individual strains, thus, knowledge of the regional population structure linked with phenotypic data such as with the drug resistance pattern could be relevant for the implementation of an effective TB control program tailored to specific genotypes and local circumstances [13]. Further, molecular epidemiological studies have been instrumental to define recent transmission dynamics in various settings as well as to describe the local and global population structure of the MTBC [13]. Modern molecular DNA fingerprinting methods like Mycobacterial Interspersed Repetitive Unit-Variable Number Tandem Repeats (MIRU-VNTRs) typing and spacer oligonucleotide typing (spoligotyping) are highly suited to investigate both, the population structure and transmission of the MTBC in communities or special settings such as prisons [14].

To date, no molecular epidemiological studies have been carried out to decipher MTBC strain diversity in prisons in Ethiopia. Only few studies investigated the population structure of MTBC strains in communities of central and northwestern parts of the country by spoligotyping. Due to the limited discriminatory power of this method, however, these studies reported the "ill defined" T linage as predominant strain [15–17]. There are two studies in which the combined methods of MIRU-VNTR and spoligotyping were applied, and in which Delhi/CAS was the dominating MTBC lineage [18, 19]. These two studies included only the north-western part of Ethiopia.

In order to contribute to a more comprehensive knowledge on MTBC strain diversity in the whole of Ethiopia, we conducted a pilot study to determine the population structure and drug resistance pattern of MTBC strains isolated from prisons and communities of southwestern, southern and eastern Ethiopia by combined application of MIRU-VNTR and spoligotyping. We further investigated to which extent the isolated strains were related to each other by the calculation of clustering rates.

Methods

MTBC strain collection at prisons and hospitals

Ethiopia is administratively organized within nine regional states and two federal cities. Oromia and Southern Nations, Nationalities and Peoples Regional State (SNNPRS) are among the three biggest regions with a total population of approximately 31,294,992 and 17,359,008 respectively [20]. Harari is the smallest regional state in Ethiopia with a population of about 210,000 [20]. Somali regional state and Dire Dawa city have a population of 5,148,989 and 387,000 respectively [20]. These four regional states and one city together cover an area where almost 65% of the total Ethiopian population resides [20]. As per Federal Ministry of Health and Health Related Indicators report of 2012/2013, TB incidence per 100,000 population in the studied regional states and city was between 258.6 (SNNPRS) and 274.7 (Dire Dawa City), respectively, per year [21].

From January 2013 to December 2013, a cross-sectional study on TB-prevalence and risk factors was conducted in 13 zonal prisons in the following regional states which are located in the Southern, South western and Eastern part of Ethiopia: Oromia, Southern Nations Nationalities and Peoples Regional State (SNNPRS) and Harari. The MTBC strains which were isolated from the sputum of symptomatic prisoners were included in this study [7]. According to the Ethiopian prison system organization, Oromia regional state had 37 (17 zonal and 20 district) prisons, while SNNPRS had 23 (13 zonal and 10 district) prisons. Harari regional state had only one zonal prison [22]. Zonal administrative prisons are the largest prisons in Ethiopian context. Most of the inmates incarcerated in zonal prisons are originated from the populations living in the respective zones. Seven out of 17 and five out of 13 prisons were selected like in a lottery from Oromia and SNNPRS, respectively, while the only prison of Harari regional state was included in the study. By this approach, ca. 35% of the total prison population of the included regional states was represented in this study. Briefly, we applied the WHO questionnaire [23] to screen up 15.495 prison inmates for the presence of TB symptoms. Two sputum samples were collected from those inmates who fulfilled the criteria for a person presumed to have TB. One sample was processed for direct smear microscopy performed at the prisons, the second sample was used for MTBC culture performed in the Jimma University Mycobacteriology laboratory. Further details on study population, methodology of sample processing and data collection as well as research outcome have been described previously by Ali et al. [7].

From August 2013 to December 2013, all MTBC strains which were routinely collected from newly diagnosed, smear positive pulmonary TB patients diagnosed

in Jimma, Nekemtie, Ambo, Yabelo, Mizan, Dire Dawa, Harar and Jigjiga at ambulant health care centers or hospitals were included in this study. One early morning sputum was collected per patient and analyzed with smear microscopy in TB laboratories which are linked to hospitals in the above named cities which are located in the regional states Oromia, SNNPRS, Harari, Somali and Dire Dawa. All laboratory procedures were performed by trained hospital staff. The remaining was temporarily stored in a refrigerator until transportation to the Jimma University Mycobacteriology Laboratory. Socio-demographic and previous treatment history data were extracted from the registration book of tuberculosis clinics in respective hospitals.

Laboratory methods

All sputum samples, from both prisoners and community based TB patients, were cultivated on LJ (BBL™ Lowenstein-Jensen Medium) at Jimma University Mycobacteriology Laboratory and afterwards transported to Research Center Borstel (RCB), Germany, for further analysis. At RCB, the strains were first reactivated on liquid mycobacterium growth indicator tube system (MGIT) 960. Drug susceptibility testing (DST) was performed using the MGIT SIRE kit at a critical concentration of streptomycin (STM) 1 μg, INH 0.1 μg, RIF 1 μg and ethambutol (EMB) 5 μg as previously described by the manufacturer [24]. DNA was extracted from all isolates for following molecular analyses, including genotyping methods [18]. Spoligotyping and 24- loci MIRU-VNTR analysis was performed as described previously [25, 26], for MIRU-VNTR typing customized kits were used (Genoscreen, Lilli, France). Spoligotypes common to more than one strain were designated as shared types (ST) and was assigned a shared international type number (SIT) according to the updated version of the international spoligotype database SpolDB4 [27]. MIRU-VNTR profiles with double alleles at a single locus were considered to represent heterogeneous populations of the same strain, whereas those with double alleles at 2 or more loci were considered to represent mixed infections or to indicate cross-contamination.

Basic strain classification and MLVA MTBC 15–9 nomenclature assignment was done using the MIRU-VNTR*plus* database [28, 29]. For the clustering analysis, samples with complete spoligotyping and MIRU-24 results were included. Cluster was defined as two or more MTBC isolates sharing identical MIRU-24 and spoligotyping patterns. Heterogeneous isolates with double alleles at only one locus were included in the cluster analysis (both patterns were compared). Isolates with no PCR amplicon at only one locus were treated as missing data at the respective locus and also were included, whereas isolates lacking amplicons at two or more loci were excluded [28, 30].

The molecular typing data was analyzed with the Bionumerics software (version 7.5; Applied Maths, Sint-Martens-Latem, Belgium) as recommended by the manufacturer. A dendrogram was generated using the unweighted pair group method with arithmetic averages (UPGMA) based on the copy number of 24-loci MIRU-VNTR. The UPGMA tree was further processed using EvolView [31].

Statistical analysis

Data were analyzed by STATA software version 10.0. The distribution of proportions of categorical variables were compared using chi-square or Fisher's exact test, were appropriate. Logistic regression modeling was performed to estimate the crude effect of several risk factors on clustering by comparing their association with unique isolates versus clustered isolates. Those risk factors which were significantly associated with clustering in the crude analysis were included in the multivariable regression model. *P*-values <0.05 were considered as statistically significant.

Results

A total of 127 MTBC strains were initially isolated from sputum specimens collected in prisons (24) and hospitals (103). Out of these, 18 (14.17%) isolates were excluded from the final analysis: ten strains could not be reactivated in (liquid) culture, five isolates were mixed infections and three isolates had repeatedly inconsistent DST results.

The basic information on the 109 participants and strains included in the final analysis are depicted in Table 1. As expected, there was a significant gender difference between participants from prisons, where 90.48% were male, and hospitals with 51.14% males ($p < 0.001$). The mean age of the study subject was 29.03 (95% CI; 26.89, 31.2) with no significant difference between prison inmates and hospital participants (t = 0.93). All 88 participants from hospitals and 17 out of 21 (80.95%) inmates from prison had never received previous TB therapy. MTBC strains from prison inmates were collected in Oromia and Southern Nations, Nationalities and Peoples Regional State (SNNPRS) regional states. TB isolates from hospital patients were additionally collected in Somali regional state and Dire Dawa city administration.

MTBC population structure

Based on combined 24-loci MIRU-VNTR and spoligotyping patterns all 109 isolates could be classified into 11 previously described lineages and sub-lineages. The majority (27.52%) of the strains were Ethiopia_H37RV like, followed by Ethiopia_3 (16.51%) and Delhi/CAS (16.51%) (Table 1). Seventeen isolates (15.60%) were assigned to the Euro-American Superlineage. One (0.92%) strain of the Beijing lineage was found. There was no statistical

Table 1 Basic participant information and strain characteristics

Variable	Prison, % (n/N)	Hospital, % (n/N)	*p*-value	Total% (n/N)
Sex				
Male	90.48(19/21)	51.14(45/88)	0.001	58.72(64/109)
Age				
> 45 years	14.29(3/21)	7.95(7/88)	0.366	9.17(10/109)
Previous TB treatment				
No	80.95(17/21)	100.00(88/88)	NA	
Regions				
Oromia	33.33(7/21)	10.23(9/88)		14.68(16/109)
SNNPRS	66.67(14/21)	39.77(35/88)		44.95(49/109)
Dire Dawa	–	11.36(10/88)	0.001	9.17(10/109)
Harar	–	15.91(14/88)		12.84(14/109)
Somali	–	22.70(20/88)		18.35(20/109)
Lineage				
Delhi/CAS	14.29(3/21)	17.05(15/88)		16.51(18/109)
Ethiopia_H37Rv like	42.86(9/21)	23.86(21/88)		27.52(30/109)
Euro-American Superlineage	14.29(3/21)	15.91(14/88)		15.60(17/109)
LAM	4.76(1/21)	3.41(3/88)		3.67(4/109)
Ethiopia_3	19.05(4/21)	15.91(14/88)		16.51(18/109)
Haarlem	–	9.09(8/88)	0.784	7.34(8/109)
Ural	4.76(1/21)	5.68(5/88)		5.50(6/109)
Lineage 7	–	3.41(3/88)		2.75(3/109)
EAI	–	2.27(2/88)		1.83(2/109)
X-type	–	2.27(2/88)		1.83(2/109)
Beijing	–	1.14(1/88)		0.92(1/109)
Clustering				
Yes	28.57(6/21)	31.82(28/88)	0.773	31.19(34/109)
Drug resistance				
Streptomycin[a]	20.00(4/20)[c]	14.94(13/87)	0.577	15.89(17/107)
Isoniazide	9.52(2/21)[b]	6.82(6/88)	0.669	7.34(8/109)
Rifampicin	9.52(2/21)[b]	4.55(4/88)	0.369	5.50(6/109)
Ethambutol[a]	10.00(2/20)[b]	3.41(3/88)	0.205	4.63(5/108)
MDR-resistance	9.52(2/21)[b]	2.27(2/88)	0.112	3.67(4/109)
Any drug resistance	19.05(4/21)[c]	21.59(19/88)	0.797	21.10(23/109)

N = total number of strains isolated from prisons (21) and hospitals (88)
n = number of strains in the specific subgroup
NA = not applicable
[a]note missing values
[b]includes one subject with TB in the past
[c]includes two subjects with TB in the past

difference observed between prison isolates and hospital isolates in relation to strain diversity although Haarlem, EAI, Lineage 7, X-type and Beijing lineage were not isolated in prisons ($p = 0.784$) (Table 1). The different lineages of the isolates are depicted in a radial UPGMA tree based on MIRU-VNTR analysis (Fig. 1).

The combined UPGMA tree including the Ethiopian MTBC strains of this study and those of a previous study performed by our group in the northwestern part of the country [18] is presented in the online supplement (Additional file 1: Figure S1). Interestingly, the frequency of particular genotypes is variable if different areas in the country are considered.

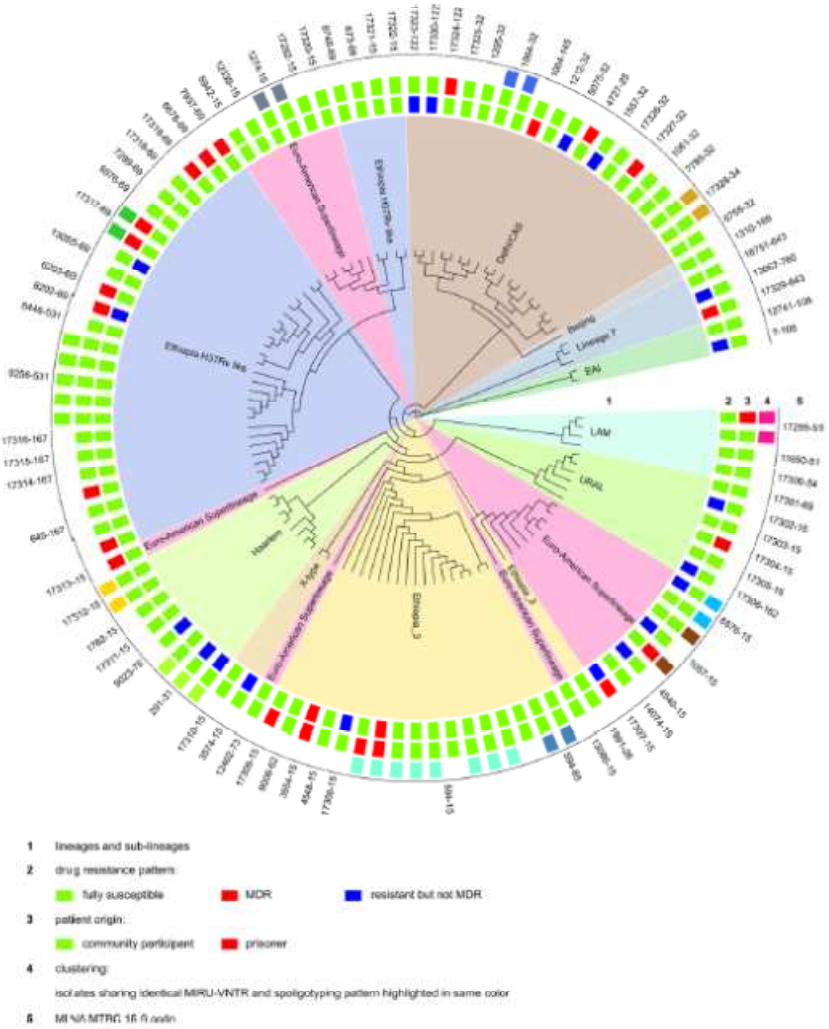

Fig. 1 Radial UPGMA tree based on the copy numbers of MIRU-VNTR 24-loci. *Inner circle*: lineages and sub-lineages, (EAI - East African Indian, LAM - Latin American Mediterranean, CAS - Central Asia). Small rectangles in the second circle: drug resistance pattern of the individual isolates (*green* - fully susceptible, *red* - MDR, *blue* - resistant but not MDR). Small rectangles in the third circle: origin of the patient (*green* - community participant, *red* - prisoner). Small rectangle in the fourth circle: clustering according to 24-loci MIRU-VNTR and spoligotyping pattern analysis; isolates with identical genotyping profile are highlighted in same color. *Outer circle*: MLVA MTBC 15–9 code

Drug resistance profile

In total, 27 (24.77%) isolates were resistant to at least one anti-tuberculosis drug (Table 1, Fig. 1). The highest mono-resistance detected was against STM (15.89%), followed by INH (7.34%), RIF (5.50%) and EMB (4.63%). The overall MDR rate was 3.67% (4 out of 109). MDR-prevalence among community isolates was 2.27% (2 out of 88) whereas it was 9.52% in prison isolates (2 out of 21, with one isolate from an inmate with previous history of TB) ($p = 0.112$) (Table 1).

Among identified lineages, 1 of 3 of Lineage 7 isolates, 11.11% (2 of 18) of Ethiopia_3 isolates and 5.56% (1 of 18) of Delhi/CAS isolates were MDR TB strains (Table 2).

Cluster analysis

Based on 24 loci MIRU-VNTR and spoligotyping analysis 34 of 109 (31.2%) isolates were grouped into 12 different clusters ranging from 2 to 8 strains in size, with the largest cluster comprising 8 strains of the Ethiopian_3 lineage, followed by Ethiopia_H37Rv like (7 strains) and Euro-American Superlineage (6 strains) (Fig. 1 and Table 3). Among strains isolated from prisons 28.57% were clustered, which was not significantly different from the

Table 2 Anti TB drug resistance and MDR pattern by lineages

Lineage (N = 109)	STM n (%)[b]	INH n (%)[b]	RIF n (%)[b]	EMB n (%)[b]	MDR n (%)[b]
Delhi/CAS (18)	4(23.53)	1(5.56)	1(5.56)	1(5.56)	1(5.56)
Ethiopia_H37Rvlike (30)	2(6.90)	1(3.33)	0	0	0
Euro-American Superlineage (17)	4(23.53)	0	0	0	0
LAM(4)	0	0	0	0	0
Ethiopia_3 (18)	2(11.11)	2(11.11)	3(16.67)	2(11.11)	2(11.11)
Haarlem (8)	3(37.50)	0	0	0	0
Ural(6)	0	1(16.67)	0	0	0
Lineage 7(3)	2	2	1	2	1
EAI (2)	0	0	1	0	0
X-type (2)	0	1	0	0	0
Beijing (1)	0	0	0	0	0
Total (109)[a]	17/107(15.89)	8/109(7.34)	6/109(5.50)	5/108(4.63)	4/109(3.67)

N = total number of strains
n = number of strains in the specific subgroup
[a]note missing data for STM and EMB
[b]Percentage was calculated for those lineages with more than 5 strains

proportion (31.82%) of clustered strains collected in the community, ($p = 0.773$) (Table 1). Two strains isolated from prisoners were clustered with two strains from community members (Fig. 1). The remaining 75 isolates did not share their 24 loci MIRU-VNTR and spoligotyping patterns with any other isolate and are considered unique.

We analyzed potential risk factors for clustering in this study and found that the region from where a strain was collected and the lineage type were independently associated with clustering of TB strains (Table 3). For example the odds for clustering of TB strains from Somali region were more than eight times higher compared to those from Oromia region (Table 3). Further, lineages of Ethiopia_3 and also Haarlem were significantly associated with clustering (Table 3). In this study, drug resistance as well as demographic characteristics (age and sex) was not a risk factor for clustering.

The combined cluster analysis of the isolates collected in our current study and those of Tessema et al. showed eight clusters comprising isolates from both studies (Additional file 1: Figure S1). Those clusters were formed by strains belonging to the Delhi/CAS, Ethiopian_H37Rv like, Haarlem and Ethiopia_3 lineage.

Discussion

In this study, we analyzed the MTBC strain population in prisons and communities of southern, southwest and eastern Ethiopia using combined spoligotyping and MIRU-VNTR typing methods. In agreement with results of previous reports from Ethiopia we could also show a high strain diversity in our study [19, 25, 32]. About 34% of the MTBC strains analyzed are Ethiopian specific Lineages and sub-lineages, Lineage 7, Ethiopia_H37Rv like and Ethiopia_3, which were described recently and have not yet been reported elsewhere [18, 19, 33]. Opposed to other studies conducted in northwest Ethiopia Delhi/CAS was not the dominating lineage [18, 19]. Tessema et al. hypothesized that the influx of Indian and Chinese peoples to Ethiopia due to growing business relations with Ethiopia introduced the Delhi/CAS lineage in the country [18]. If this hypothesis was true, the Delhi/CAS lineage dominancy might have started from the center Addis Ababa and is now processing to the periphery. This could explain why in some remote areas investigated in this study, where a relevant proportion of the population are still leading a nomadic life style which is driven by the search for water and grazing land for their cattle, not Delhi/CAS but the Ethiopian lineages are still dominating.

Clustering is a marker of recent transmission [34, 35] and knowing the clustering rate of TB strains that circulate in the community can help to evaluate the performance of TB control programs or to formulate new control strategies. The overall clustering rate in this study was 31.19% which was lower than the previously reported 45.1% from Ethiopia [18]. On the other hand our clustering rate is consistent with the 32% reported for Amhara regional state study [19]. This data could suggest that a relevant proportion of active TB cases were due to reactivation of latent infection. Indeed, there was a significant decline in TB prevalence observed in Ethiopia in the past five years [36]. This decline in active TB cases might have contributed to the lower clustering rate seen in this study compared to older reports. The cluster analysis of our strains collected in 2013 and strains analyzed by Tessema et al. in 2009 revealed several clusters, including strains from both studies, indicating that some strains remain in the population

Table 3 Geographical information, phylogenic lineage, drug resistance pattern, and their association with strain clustering

Variables	Unique strains (n)	Clustered strains (n)	COR (95% CI)	*p*-value*	AOR (95% CI)	*p*-value$
Regions						
Oromia	14	2	1			
SNNPRS	32	17	3.72(0.76,18.31)	0.11	6.19 (0.92,41.83)	0.06
Dire Dawa	7	3	3.00(0.40,22.30)	0.28	3.98(0.35,44.90)	0.26
Harar	11	3	1.91(0.27,13.50)	0.52	1.36(0.15,12.70)	0.79
Somali	11	9	5.73(1.02,32.10)	0.04	8.76(1.07,71.88)	0.04
Lineage						
Delhi/CAS	14	4	1			
Ethiopia_H37Rv like	23	7	1.07(0.26,4.31)	0.93	1.20(0.28,5.20)	0.81
Euro-American Superlineage	11	6	1.91(0.43,8.48)	0.40	1.76(0.37,8.41)	0.48
LAM	2	2	3.50(0.37,33.31)	0.28	5.46(0.46,64.04)	0.18
Ethiopia_3	8	10	4.38(1.03,18.63)	0.05	8.72(1.69,45.02)	0.01
Haarlem	3	5	5.83(0.95,35.72)	0.06	7.88(0.98,63.23)	0.05
Others	14	0	-	-		
Streptomycin						
Resistant	13	4	1			
Susceptible	61	29	1.55(0.46,5.150)	0.48	NA	NA
Isoniazide						
Resistant	7	1	1			
Susceptible	68	33	3.39(0.40,28.76)	0.26	NA	NA
Rifampicin						
Resistant	5	1	1			
Susceptible	70	33	2.36(0.26,20.98)	0.44	NA	NA
Ethambutol						
Resistant	4	1	1			
Susceptible	70	33	1.89(0.20,17.54)	0.58	NA	NA
MDR						
Yes	3	1	1			
No	72	33	1.37(0.14,13.72)	0.79	NA	NA
Any resistance						
Yes	19	4	1			
No	56	30	2.54(0.79,8.16)	0.12	NA	NA

n = numbers of clustered (i.e., isolates sharing identical MIRU-24 and spoligotyping patterns with at least one other isolate) or unique (i.e., isolates that do not share their MIRU-24 and spoligotyping pattern with any other isolate) strains in each stratum
COR = crude odds ratio
AOR = adjusted odds ratio: significant variables in the crude analysis were adjusted for age, region and genotype
NA = not applicable
* = *p*-value for univariate regression model
$ = *p*-value for multivariate regression model

and lead either to reactivation of a remote infection or are effectively transmitted over a four years timeframe in Ethiopia [18]. Interestingly, transmission rates of active TB seemed to be higher in Somali regional state. Strains from this regional state showed a significantly higher clustering rate compared to other regional states. This finding could be explained with the geographic context of Somali regional state which is bounded by Djibouti, Somalia and Kenya [37] where a free and intensive movement of peoples living in the border areas might have contributed to an ongoing TB transmission.

As there was no statistical difference between the clustering rates in the communities (31.82%) and prisons (28.57%), our data suggest that transmission rates are not higher in prisons than in communities. This comparison, however, is hampered by the slightly different

study regions, the low number of MTBC strains isolated from prison inmates and the different approaches and coverage of MTBC strain collection in prisons and communities. On the other hand we could show that MTBC strains which were isolated in prisons build clusters with strains collected from community members. This finding could indicate that TB infection was acquired in the community and developed later to active TB disease under the specific conditions found in prisons. This hypothesis is further supported by findings which were previously published by our group [7]. In that specific study, a contact with a TB case at home before incarceration was one of the strongest risk factors for active TB disease in prison [7].

Despite the great achievements seen in Ethiopia to reduce overall TB prevalence [1], the control of MDR-TB still seems a distant prospect. In this study we have observed an MDR-TB prevalence in the community of 2.27% which was consistent with the 2.3% of the Ethiopian public health institute (EPHI) survey report performed in year 2014 [3]. However, comparing our findings with 0.8% seen in 2002 [38] and the 1.6% estimation of WHO in 2011 indicates that MDR-TB is increasing with alarming rate through time [3, 39]. In prisons, the MDR TB prevalence (9.52%) and also the number of subjects with TB in the past was higher than in communities, although, this difference was not statistically significant in our analysis of a limited number of isolates. This result is alarming since prisons may act as a reservoir for MDR-TB in the country. The occurrence of a single MDR-TB case in prison might have huge implications for prison health and the community, considering bidirectional communication between both.

This study has several limitations and the findings should be interpreted with care. First, due to a relative short study period, the number of MTBC isolates in this study, specifically the number of strains from prisoners, is lower than in other reports [18, 19]. Therefore, the magnitude of clustering and also specific risk factors on clustering, e.g., drug resistance, could have been undetected or underestimated. Second, only TB strains from selected communities (which had access to a TB laboratory located in neighboring hospitals) and prisons (to which the investigators had permission to enter) were included in the analysis. This fact could introduce selection bias, especially as only characteristics of TB strains from participants who had access to the health system or who were inmates in the rather huge zonal prisons could be studied. Third, clustering rates could be imprecise and rather reflecting the spread of dominant strains types but not recent transmission as not all MTBC strains in the study areas were analyzed but only those collected in the catchment area of large hospitals and large prisons. Fourth, the rather cross-sectional study approach in both prison and community settings did not allow for the investigation of risk factors of TB transmission in cases with an incubation period of more than 12 or five months, respectively. Fifth, HIV test results were not available for community based patients. This hampered the analysis of the influence of HIV on TB transmission and clustering. Finally, this pilot study highlights the need to further investigate the drug resistance, population structure and transmission dynamics of TB in communities and prisons in Ethiopia as well as the interaction of both groups in a larger and prospective survey.

Conclusion

Our study provides first data on MTBC population structure and drug resistance pattern of strains found in Ethiopian prisons and in regional states of the country which were not studied before. Our findings suggest that TB is still not sufficiently controlled in specific, potentially remote, areas of the country and highlight the need for improved tools and new strategies aiming for MDR-TB control, especially in prisons. The fact that TB strains from prisoners are forming clusters with community based TB strains is worth noting and stresses the importance of the inclusion of prisons in strategies for TB control in the whole of Ethiopia. Future studies of sufficient duration and area-wide strain collection need to be performed to improve our knowledge on risk factors for TB transmission in Ethiopia.

Additional file

Additional file 1: Figure S1. Radial UPGMA tree based on the copy numbers of MIRU-VNTR 24-loci of 109 isolates of the current study and additional 240 isolates from Tessema et al. Inner circle: lineages and sub-lineages (EAI - East African Indian, LAM - Latin American Mediterranean, CAS - Central Asia). Small rectangle in the second circle: affiliation of the isolate (green - current study, red - Tessema et al. northwest Ethiopia). Small rectangle in the third circle: drug resistance pattern (green - fully susceptible, red - MDR, blue - resistant but not MDR). Small rectangle in the outer circle: clustering according to 24-loci MIRU-VNTR and spoligotyping pattern analysis; isolates with identical genotyping profile are highlighted in same color. (PNG 2468 kb)

Abbreviations

DST: Drug susceptibility testing; EMB: Ethambutol; INH: Isoniazide; MDR: Multi-drug resistance; MDR-TB: Multi drug resistance tuberculosis; MGIT960 system: *Mycobacterium* growth indicator tube system; MIRU-VNTRs: Mycobacterial interspersed repetitive unit-variable number tandem repeats; MLVA: Multiple locus variable number tandem repeat analysis; MTBC: *Mycobacterium tuberculosis* complex; RIF: Rifampicin; SIT: Shared international type number; SNNPRS: Southern Nations Nationalities and Peoples Regional State; STM: Streptomycin; TB: Tuberculosis; UPGMA: Unweighted pair group method with arithmetic averages

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Availability of data and materials
All the data in relation to this research is included in this manuscript and additional files.

Authors' contributions
Conceived and designed the experiments: SA, AH, AR, MH, TL. Perform the experiments: SA, PB, AH, AR, AW, SN, MH. Analyzed the data: SA, PB, AR, SN. Contributed reagents/material/analysis tools: SA, PB, AH, AR, AW, SN, NH, MP, TL, MH. Wrote the paper: SA, PB, AH, AR, SN. All authors contributed comments on the manuscript and agreed with the final version. 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
This study was approved by Jimma University Ethical Board ref number; RPGC/04/2005/2012 and CIH[LMU] ethical board (Ref 251–13). Written informed consent from each prison participant was obtained before sputum and data collection. Written permission to use the remaining sputum sample of community based smear positive pulmonary TB patients and accessing the registration book for extraction of routine data (age, sex, treatment history and geographic origin) was granted from each individual health institution officials. Patients were informed about the study and that their privacy will be respected and no personal data will be published.

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9. Limitations of the study

This research has some limitations. Due to adoption of more stringent WHO recommended TB in prison screening approach and logistics constraints to employ chest x-ray, the observed prevalence of TB in Prison might be under estimated. Furthermore, relatively low number of M. tuberculosis strains isolated from prison might have influenced the clustering and transmission dynamics of *M.tuberculosis* in prison. Apart from these, the thesis is the first well organized, structured and representative research done in Ethiopia prisons so far. It surfaced out the magnitude, distribution, determinants and lineage diversity of *M.tuberculosis* in Ethiopia prison. It also has indicated the areas of priorities and strategies that would be used for TB control in Ethiopian prison and the community as well.

10. Conclusion and recommendation

The average TB prevalence in prison inmates is twice higher than the prevalence in the general population and a great variability of prevalence among different prisons existed. This variability and the higher TB burden in prisons located far from the capital suggest that the national TB control measures are either not similarly implemented in the different prisons or have a differing impact on TB-control in specific prison environments or study populations. This needs further attention and future studies should focus on risk factors related to the individual but also on factors inherent to the general population and the prison environment including the functioning of TB control strategies.

Tuberculosis in communities and prisons is caused by a variety of MTBC lineages with predominance of local Ethiopian lineages. The observed overall clustering rate is low, indicating reactivation of a remote infection rather than new transmission. The increasing prevalence of MDR is alarming. These findings suggest the need for new approaches to address latent tuberculosis infection and to control MDR-TB in Ethiopia.

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List of figures

Abbreviations

ACP:	Acyl Carrier Protein
AFB:	Acid Fast Bacilli
AG:	Arabinogalactan
BCG:	Bacillus Calmette Guerin
CD:	Cluster of Designation
CNS:	Central Nervous System
CRISPRS:	Clustered Regularly Interspaced Short Palindromic Repeats
DNA:	Deoxyribonucleic Acid
DOTS:	Directly Observed Treatment Short course
DST:	Drug Susceptibility Testing
E:	Ethambutol
FDA:	Food and Drug Administration Agency
H:	isoniazid
HIV:	Human Immuno Deficiency Virus
HLA:	Human Leukocyte Antigen
IFN-γ:	Interferon Gamma
IGRA:	Interferon Gamma Releasing Assay
INH:	Isonicotinic acid Hydrazide
IS3:	Insertion Sequence 3
IS6110:	Insertion Sequence 6110
LAM:	Lipoarabinomannan
MB:	Mega Base
MDR-TB:	Multi Drug Resistance Tuberculosis
MGIT 960:	Mycobacterium Growth Indicator Tube system 960
MHC:	Major Histocompatiblity Complex
MIRY-VNTRs:	Mycobacterial Interspersed Repetitive Unit-Variable Number Tandem Repeats
MOT:	Mycobacterium Other than Tuberculosis

MTBC:	Mycobacterium tuberculosis complex
NTM:	Non Tuberculosis Mycobacterium
PCR:	Polymerase Chain Reaction
PG:	Peptidoglycan
PPD:	Purified Proteins Derivative
PuvII:	Proteus vulgaris II restriction enzyme
PZA:	Pyirazinamide
R:	Rifampicin
RDs:	Region of Differences
RFLP:	Restriction Fragment Length Polymorphism
RIF:	Rifampicin
RpsA:	Ribosomal Protein S1
SNNPRS:	Southern Nations, Nationalities and Peoples Regional State
SpolDB4:	Spoligotyping database
Spoligotyping:	Spacer oligonucleotide typing
TB:	Tuberculosis
TbD1:	Tuberculosis Deletion one
TH:	T Helper Cells
TLR:	Toll-Like Receptor
TST:	Tuberculin Skin Test
WHO:	World Health Organization
XDR-TB:	Extensively drug-resistant tuberculosis
Xpert:	GeneXpert
Z:	Pyrazinamide