

Spatial Clustering and Genetic Diversity of Mycobacterium Tuberculosis Isolate Among Pulmonary Tuberculosis Suspected Patients, Arsi Zone, Ethiopia

Ketema Tafess (✉ ttafess@gmail.com)

Department of Medical Laboratory Science, College of Health Science, Arsi University

Teresa Beyen

Department of Public Health, College of Health Science, Arsi University

Sisay Girma

Department of Biomedical Science, College of Veterinary Medicine and Agriculture, Addis Ababa University

Asnakech Wondimu

Disease prevention, Training and Research Unit, Federal Prison General Hospital

Gilman SIU

Department of Health Technology and Informatics, The Hong Kong Polytechnic University

Research Article

Keywords: Mycobacterium tuberculosis, spatial clustering, genotyping

Posted Date: December 4th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-113163/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Spatial clustering and Genetic Diversity of *Mycobacterium Tuberculosis* Isolate among Pulmonary Tuberculosis Suspected Patients, Arsi Zone, Ethiopia.

Ketema Tafess^{1*}, Teresa Kisi Beyen², Sisay Girma³, Asnakech Girma⁴, Gilman SIU⁵

Author affiliation :

¹Department of Medical Laboratory, College of Health Sciences, Arsi University, Asella, Ethiopia

² Department of Public Health, College of Health Sciences, Arsi University, Asella, Ethiopia

³Department of Biomedical Science, College of Veterinary Medicine & Agriculture, Addis Ababa University, P.O.Box 34 Bishoftu, Ethiopia.

⁴Disease Prevention and Research Division, Federal Prison General Hospital, Addis Ababa

⁵Department of Health Technology and Informatics, The Hong Kong Polytechnic University, Hong Kong, Hong Kong

Corresponding Author

Ketema Tafess

Email: ttafess@gmail.com

Phone: (251) 901238022

Author initial and email

Ketema Tafess (KT): ttafess@gmail.com

[Teresa Kisi Beyene \(TKB\): terek7@gmail.com](mailto:Teresa Kisi Beyene (TKB): terek7@gmail.com)

Sisay Girma (SG): girmasis@gmail.com

Asnakech Girma Wondimu(AGM): girmaasni@gmail.com

Gilman SIU (GS): gilman.siu@polyu.edu.hk

Abstract

Background: Tuberculosis remains a serious public health concern globally. The enormous social, economic and health impacts of the diseases are attributed to the lack of detailed understanding of the prevalence, geospatial distribution, population structures, and genotypic variants of the circulating *M. tuberculosis*.

Methods: Structured questionnaire, mycobacterial culture, and standard 24-Mycobacterial Interspersed Repeated Units-Variable Number Tandem Repeats (MIRU-VNTR) were employed to collect sociodemographic characters, residence linked information, and genotype the isolates. The retrospective discrete Bernoulli model was used to identify the hot spot districts of sputum smear positivity, and Web-based *Miru-VNTRPlus* were used for the analysis of the data.

Results: Out of 832 presumptive pulmonary tuberculosis (PTB) suspects, 119 (14.3%) were smear-positive. In the multivariate binary logistic model, PTB suspected patients in the age groups of ≤ 25 and 25-34 and those from rural residents were 4.53 [AOR=4.53; 95%CI: 2.25-9.13], 3.00 [AOR=3.00; 95%CI:1.41-6.35] and 1.65 [AOR=1.65; 95%CI: 1.01-2.70] times at higher risk of turning smear-positive. Eleven (47.8%) districts of Arsi Zone were shown to have high rate of clustering (RR = 2.27; 95% CI: 1.62-3.2) of smear-positive PTB. Of 72 isolates queried for the lineage assignment, 59 (81.9%) were classified into the previously known lineages and 13 (18.1%) were not assigned to any known lineages. Overall, 42 (58.3%) belong to *M. tuberculosis* lineage 4 (Euro-American), 16 (22.2%) *M. tuberculosis* lineage 3 (Delhi/CAS), and 1 (1.4%) *M. tuberculosis* Lineage 1 (Indo-Oceanic/ East Africa Indian). Further classification to the sublineage indicates that the predominant lineage was Delhi/CAS comprising 16 (22.2%) isolates followed by 15 (20.8%) isolates belonging to Haarlem. The remaining isolates were distributed as 13 (18.1%) TUR, 6 (8.3%) LAM, 4 (5.5%) URAL, 4 (4.5%) NEW-1 and 1(1.4%) EAI.

Conclusion: Our study showed higher smear-positive results among PTB suspected patients and remarkable spatial variation across districts of Arsi Zone in smear-positive PTB. This information together with the genotypic features could be used as input for the efforts of designing control strategies.

Keywords: *Mycobacterium tuberculosis*, spatial clustering, genotyping

Introduction

Tuberculosis (TB) remains one of the major public health problems with an estimated 10 million new cases and 1.6 million deaths globally [1]. Ethiopia is 7th among the 30 high-burden TB countries with an annual incidence rate of 164 cases/10,000 population. Despite the several efforts and achievements in the control of TB, the disease is among the major public health problems in the country with an estimated mortality of 24 cases/10,000 population. One of the major reasons for the unabated TB challenge is the uneven distribution of the disease across the country [2]. Particularly, the heterogeneous prevalence of smear-positive TB in small geographic areas such as districts substantially affects the transmission dynamics and control efforts.

Previous epidemiological and spatial-temporal studies conducted in various parts of the country showed that there is a significant geographic variation of tuberculosis burden across various administrative units of the countries such as districts and Kebeles [3, 4]. The variation could be associated with differences in the community and individual level risk factors and discrepancies in the implementation of control strategies [5]. Such small-area variations of TB may have important implications for local and national health policy, to target interventions to those areas and communities at the highest risk. However, previous spatial analyses of TB have been generally conducted using routinely collected TB notification data consisting of limited variables. Particularly those studies usually focus on secondary data from health facilities and lack integrating the defining feature of heterogeneity of tuberculosis burden such as bacterial genetic characters.

The geographic variability of the TB burden is associated with host, bacterial and environmental factors [6]. The physiological and medical parameters of the host play a significant role in maintaining the transmission dynamics of TB. For example, the smear-positive pulmonary tuberculosis patients transmit *M. tuberculosis* significantly than smear-negative patients [7, 8], playing a pivotal role in the epidemiology and transmission of tuberculosis. That is why smear microscopy remains the backbone to identify the most infectious individuals despite its limited sensitivity [9]. Subsequently, the TB control program places the greatest emphasis on the early diagnosis of smear-positive PTB to ensure prompt initiation of treatment to reduce the transmission of infection.

Bacterial genetics is a powerful and well-studied factor contributing significantly to the uneven distribution of tuberculosis and disease outcomes. *Mycobacterium tuberculosis* complex (MTBC) is considered as clonal bacteria species sharing 99.9% similarity at the nucleotide level. Nonetheless, recent advances in molecular techniques and genome sequencing approaches acknowledged the existence of huge diversity among this clonal species and divided the MTBC into 7 main human-adapted lineages (L1-7) (Lineage 1 (Indo-oceanic), Lineage 2 (East-Asian), Lineage 3 (East-Africa-Indian), Lineage 4 (Euro-American), Lineage 5 (West-African 1/*Mycobacterium africanum*), Lineage 6 (West African 2), Lineage 7 (Woldia/Ethiopia) and animal-adapted lineages [10]. Lineage 2, 3, and 4 are called the modern lineages distributed across the wide geographic area whereas lineage 1, 5, and 6 are called the ancient lineages that are mainly restricted in a certain geographic area. Lineage 7 (recently named as *Aethiops vetus*) is discovered from the central highland of Ethiopia.

M. tuberculosis genotyping has several implications as the genetic variation could determine the phenotypic characteristics like disease outcomes. Genotypes of the strains affect the bacterial growth rates and gene expressions, emergence, and distribution of drug resistance, vaccine efficacy, and clinical presentations. The strain type also influences geographic distribution patterns, transmission capacities, and disease-causing capacities. For example, lineage 2 (Beijing) and lineage 4 (Euro–American) are the most widely distributed and virulent than the West African lineages [11]. Further characterization of the lineages to sub-lineage showed the distinct characteristics of the sub-lineages. From Euro-American lineage, the sub-type Haarlem strain shows higher virulence than the geographically restricted lineages [12]. Intriguingly, the characterization of *M. tuberculosis* isolates from different parts of the world like Ethiopia could also support the impact of genetic variation on disease outcomes, and the development and validation of novel diagnostic approaches.

In this study, we aimed to determine the smear positivity, the geospatial clustering, and the genetic diversity of *M. tuberculosis* isolates obtained from Arsi Zone, Ethiopia. Continuous investigation and detailed understanding of the prevalence, geospatial distribution, population structures, and genotypic variants of the *M. tuberculosis* circulating in the areas could ensure the designing and evaluation of universally effective novel control strategies.

Material and Methods

Study Setting

The study was conducted at the Asella Teaching Hospital, University hospital of Arsi University, situated in Asella Town, South-East of Addis Ababa. The hospital has 297 beds and acts as a medical referral center for a population of 3.5 million inhabitants in the Arsi Zone and its surroundings. The Zone is found in the central part of the Oromiya National Regional State and astronomically lies between 60 45' N to 58'N and 38 32 E to 40 50' E. Currently, it is divided into 25 districts: Amigna, Aseko, Bale Gasegar, Chole, Digeluna Tijo, Diksis, Dodota, Enkelo Wabe, Gololcha, Guna, Hitosa, Jeju, Limuna Bilbilo, Lude Hitosa, Merti, Munesa, Robe, Seru, Sire, Sherka, Sude, Tena, Tiyo, Ziway Dugda. Provided that Asella Teaching Hospitals is one of the oldest health facilities with better infrastructure and experienced health personnel as well as the only referral hospital in the Zone, most tuberculosis patients particularly referral cases come to the hospital from all districts.

Samples Collection, Culture and DNA isolation

A facility-based study was conducted at Asella Teaching Hospital between February 2016-August 2016. Before the sputum collection, a pretested structured interviewer-administered questionnaire was used to collect data on socio-demographic, (age, sex, residence, education), districts of the patients (≥ 7 years old), previous contact with TB cases, and facility service-related data followed by the collection of sputum samples at a spot and the next day morning for sputum smear examinations and culture as described by Forbes *et al.*, [13]. Isolation of genomic DNA (gDNA) was conducted using the Roche Cobas Amplicor extraction kit (Roche Diagnostics, USA) as previously described [14]. The DNA samples were shipped to the Laboratory of the Health, Technology and Informatics, The Hong Kong Polytechnic University for genotyping.

Genotyping using MIRU-VNTR

The PCR amplification of the 24 MIRU-VNTRs loci was conducted using 24 pairs of primers as described previously by Supply *et al.*, [15]. Briefly, amplification was conducted in 96 well plates that enabled the amplification of eight samples per 12 loci. The target loci were amplified in a total of 20 μ l mix containing variable proportion of MgCl₂, 4 μ l of Q-solution, 0.08 μ l (5U/ μ l final concentration) HotStarTaq DNA polymerase, 0.4 μ l (10mM) dNTP, 0.4 μ l forward and reverse primers (at 20 μ M final concentration), 2 μ l 10X PCR buffer, 9.1 μ l, and

2µl of DNA template. The amplification was conducted using Veriti thermal cycler set at initial denaturation at 95°C for 15 min, 40 cycles of 94°C for 1 min, annealing at 59°C for 1 min, extension at 72°C for 1:30 min, and a final extension of 72°C for 10 min. The amplified products were mixed with 6X loading dye in a proportion of 5:2 v/v, electrophoresed for 2hrs using 1.5% agarose gel (Vivantis Agarose (Molecular Biology Grade) in 1X TBE (Tris-Borate EDTA) buffer with RedSafe™ Nucleic Acid Staining Solution (iNtRON Biotechnology) and visualized under UV transillumination. The product size was compared against the 100bp ladder and the copy number was determined manually using allele calling tables. Each locus for eight isolates was electrophoresed together to reduce the bias during size determination (Supplementary figure -1).

Data Analysis

After the collected data were entered into the excel spreadsheet, it was exported to SPSS version 22 for descriptive and Binary logistic regression model analysis. For spatial analysis, using retrospective discrete bernoulli model, the data was exported to SaTScan version 9.6 and Quantum Geographical information system (QGIS) version 3.12. The binar logistic regression model was used to determine the association of patients' characteristics with sputum smear positivity, while retrospective discrete Bernoulli model was used to identify the sputum smear positivity hot spot districts. The maximum spatial cluster size were considered as 50% of the population at risk; the Boscoe's limit on cluster risk level (relative risk) of more than one was used to restrict high rate cluster. The final significant clusters were declared at a p-value less than 0.05 (i.e. which combined the Standard Monte Carlo, Sequential Monte Carlo, and Gumbel Approximation) with its 95%CI of relative risks and those which had no Geographical Overlap. The maximum replication was set at 999 of Monte Carlo Replication. Finally, sputum smear positive hot spot areas were presented by QGIS as per their level of cluster. An analysis of the MIRU-VNTR data was conducted using the browser-based software (<http://www.MIRU-VNTRplus.org>) as described by Allix-Beguec *et al.*, [16]. The numerical 24 loci copy numbers of isolates were entered into an excel spreadsheet and uploaded. Similarity matching with the standard reference strains was conducted by setting the maximum distance of 0.17 followed by phylogenetic tree classification. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based dendrogram of the phylogenetic tree was constructed. A cluster rate was calculated as the number of isolates with identical MIRU-VNTR alleles divided by the total number of test isolates. The minimum spanning tree was used to detect the clonal complex of *M. tuberculosis* as well as to assess the genetic links and clonal complexes (CC). The clonal complex was defined as *M. tuberculosis* strains with only two loci difference.

Results

Sociodemographic Characteristics, Prevalence of Smear Positive and Associated Factors

A total of 832 PTB suspected patients participated in the study. Out of this, 451 (54.2%) were male and 589 (70.8%) were rural residents. The median age of the patients was 35 years with an interquartile range of 27 and age ranged from 7 up to 86 years. A total of 324 (38.9%) of the patients were illiterate and the majority, 658 (79.1%) had no previous history of hospital admission (**Table-1**).

Table-1: Sociodemographic characteristics and prevalence of smear-positive PTB February 2016, and August 2016, at Asella Hospital, Arsi Zone, Oromia Region, Ethiopia (n = 832).

Characteristics	Total number	Percent (%)	Smear Positive (%)		Chi-square	P value
			Yes	No		
Sex						
Male	451	54.2	68 (15.1)	383 (84.9)	0.48	0.55
Female	381	45.8	51 (13.4)	330 (86.6)		
Age (years)						
<=25	264	31.7	60 (22.7)	204 (77.3)	28.26	0.000
25-34	148	17.8	24 (16.2)	124 (83.8)		
35-44	139	16.7	13 (9.4)	126 (90.6)		
45-54	101	12.1	9 (8.9)	92 (91.1)		
>=55	180	21.6	13 (7.2)	167 (92.8)		
Education						
Illiterate	324	38.9	40 (12.3)	284 (87.7)	2.10	0.55
Elementary	328	39.4	50 (15.2)	278 (84.8)		
High school	122	14.7	21 (17.2)	101 (82.8)		
College and Above	58	7.0	8 (13.8)	50 (86.2)		
Residence						
Urban	243	29.2	26 (10.7)	217 (89.3)	3.63	0.06
Rural	589	70.8	93 (15.8)	496 (84.2)		
Previous history of Hospital admission						
Yes	174	20.9	23 (13.2)	151 (86.8)	0.21	0.37
No	658	79.1	96 (14.6)	562 (85.4)		
Previous history of imprisonment						
Yes	56	6.7	7 (12.5)	49 (87.5)	0.16	0.43
No	776	93.3	112 (14.4)	664 (85.6)		
The habit of drinking raw milk						
Yes	471	56.6	68 (14.4)	310 (85.6)	0.02	0.49
No	361	43.4	51 (14.1)	403 (85.9)		

Out of 832 presumptive PTB patients, 119 (14.3%) were smear-positive with variable tuberculosis prevalence among the studied sociodemographic characteristic. Accordingly, male (15.1%), age group of less than or equal to 25 (22.7%), and rural residents (15.8%) had a higher prevalence of smear-positive PTB. Out of the variables considered in this study, only age groups stand out to be significantly associated with the smear-positive rate ($X^2=28.26$, $P=0.000$). Further analysis of the considered variables with the prevalence of smear-positive PTB showed that the age and residence of the patient were the risk factors for the smear positivity. Accordingly, people at age groups of ≤ 25 and 25-34 were 4.53 ([AOR=4.53; 95%CI: 2.25-9.13]) and 3.00 ([AOR=3.00; 95%CI:1.41-6.35]) times at higher risk of turning smear-positive compared with the old age group (≥ 55 years), respectively. Likewise, though the residence site of the patients was not identified as a risk factor on the bivariate analysis, it becomes a risk factor after adjusting for other confounders on the multivariate analysis (**Table-2**).

Table-2: Crude and adjusted odds ratio for various factors that affect the smear-positive PTB during February 2016, and August 2016, at Asella Hospital, Arsi Zone, Oromia Region, Ethiopia (n = 832).

Character		Smear Positive Yes (%)	No (%)	Crude OD	95% CI	P-value	Adjusted OD	95% CI	P-value
Sex									
	Male	26 (10.7)	217 (89.3)	0.14	0.78-1.70	0.488	1.25	0.82-1.92	0.29
	Female	93 (15.8)	496 (84.2)	1			1		
Age (years)									
	<=25	60 (22.7)	204 (77.3)	3.78	2.01-7.12	0.000	4.53	2.25-9.13	0.00
	25-34	24 (16.2)	124 (83.8)	2.48	1.22-5.01		3.00	1.41-6.35	
	35-44	13 (9.4)	126 (90.6)	1.33	0.59-2.95		1.47	0.64-3.35	
	45-54	9 (8.9)	92 (91.1)	1.26	0.52-3.05		1.38	0.56-3.38	
	>=55	13 (7.2)	167 (92.8)	1			1		
Education									
	Illiterate	40 (12.3)	284 (87.7)	1.23	0.81-1.99	0.553	1.40	0.56-3.47	0.775
	Elementary	50 (15.2)	278 (84.8)	1.47	0.83-2.62		1.10	0.47-2.57	
	High school	21 (17.2)	101 (82.8)	1.13	0.50-2.57		1.25	0.51-3.11	
	College and Above	8 (13.8)	50 (86.2)	1		1			
Resident									
	Rural	93 (15.8)	496 (84.2)	0.64	0.40-1.01	0.058	1.65	1.01-2.70	0.048
	Urban	26 (10.7)	217 (89.3)	1			1		
History of hospital admission									
	No	96 (14.6)	562 (85.4)	1.121	0.68-1.83	0.64	0.95	0.57-1.59	0.852
	Yes	23 (13.2)	151 (86.8)	1			1		
Previous history of imprisonment									
	No	112 (14.4)	664 (85.6)	1.18	0.52-2.67	0.69	1.07	0.45-2.53	0.881
	Yes	7 (12.5)	49 (87.5)	1			1		
The habit of drinking raw milk									
	No	51 (14.1)	403 (85.9)	0.97	0.66-1.44	0.89	1.01	0.67-1.51	0.961
	Yes	68 (14.4)	310 (85.6)	1			1		

Spatial Distribution Tuberculosis Patients

As illustrated in Figure-1, presumptive PTB patients visited the hospital from 23 (92%) of the currently 25 districts of Arsi Zone. The disease was significantly clustered in eleven (11) (47.8%) districts (RR = 2.27; 95% CI: 1.62-3.2) of the zone while two districts (i.e. Digaluna Tijo, and Tiyo) were identified as potential clusters of the disease (RR=1.43; 95%CI: 0.95-2.16) (**Table-3**).

Table-3: Spatial distribution of smear-positive PTB in districts of Arsi Zone during February 2016, and August 2016, at Asella Hospital, Arsi Zone, Oromia Region, Ethiopia (n=119)

Cluster type	Lists of Districts within the cluster	Number of Observed cases	Number of expected cases	RR (95%CI)	Loglikelihood Ratio	P-value
High Rate Most Likely Cluster	Amigna, Shirka, Bale Gesger, Seru, Sude, Chole, Diksis, Jeju, Tena, Robe	38	20.39	2.27 (1.62, 3.2)	9.359710	0.00069
Most likely Secondary Cluster	Digaluna Tijo, Tiyo	44	36.86	1.43 (0.95, 2.16)	1.450177	0.57
Low Rate Most Likely Cluster	Lode Hetossa	1	4.92	0.20 (0.03, 1.37)	2.655211	0.291

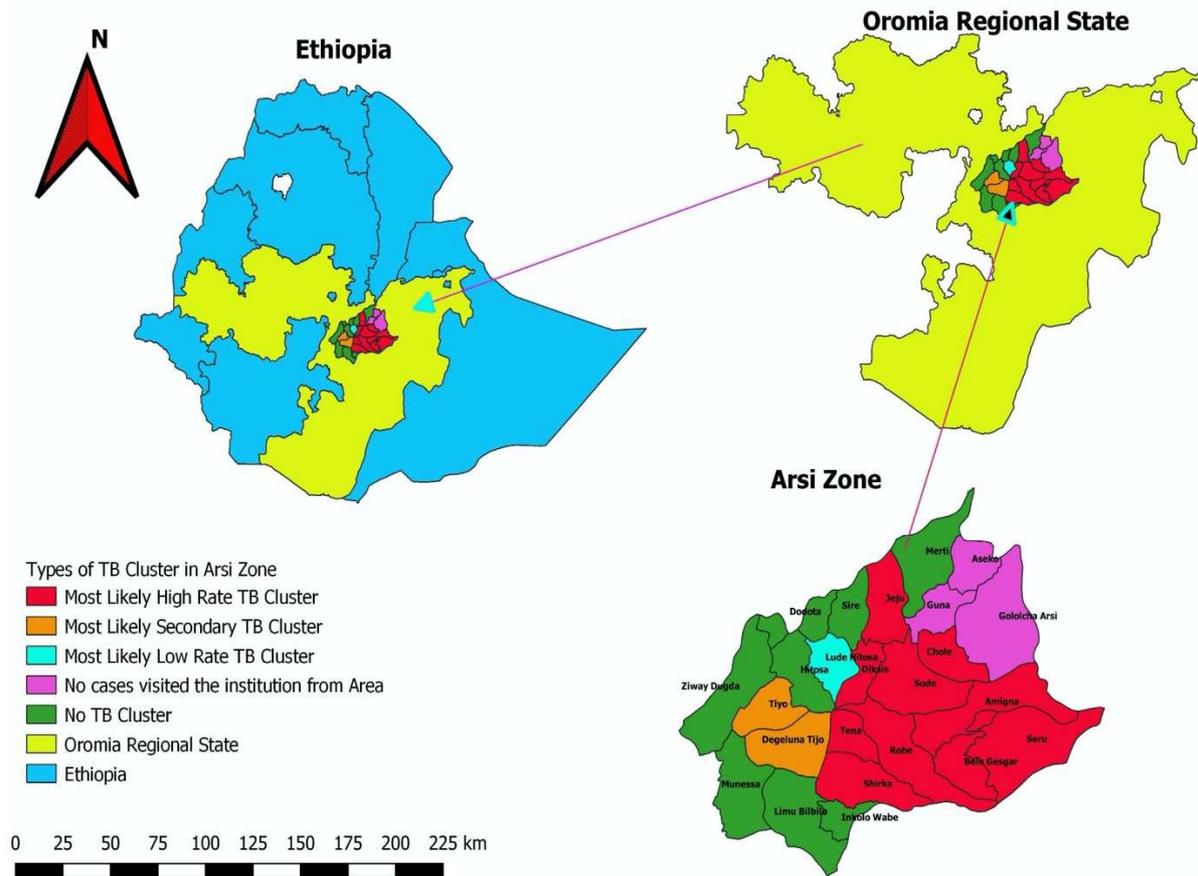


Figure-1: Spatial Distribution of smear-positive PTB in the Districts of Arsi Zone, during February 2016, and August 2016, Oromia Region, Ethiopia

Genetic Diversity of *M. tuberculosis*

Out of 90 culture positive isolates, seventy-two (72 (90%)) isolates were genotypically characterized using standard MIRU-VNTR. Of 72 isolates queried for the lineage assignment, 59 (81.9%) were classified into the previously known lineages and 13 (18.1%) were not assigned to any known lineages. Overall, 42 (58.3%) belong to *M. tuberculosis* lineage 4 (Euro-American), 16 (22.2%) *M. tuberculosis* lineage 3 (Delhi/CAS), and 1 (1.4%) *M. tuberculosis* Lineage 1 (Indo-Oceanic/ East Africa Indian). Further classification to the sublineage indicates that the predominant lineage was Delhi/CAS comprising 16 (22.2%) isolates followed by 15 (20.8%) isolates belonging to Haarlem. The remaining isolates were distributed as 13 (18.1%) TUR, 6 (8.3%) LAM, 4 (5.5%) URAL, 4 (4.5%) NEW-1 and 1(1.4%) EAI (**Table-2**). The sublineages were distributed across the districts (supplementary table-1). The constructed

phylogenetic tree exhibited a distinct clustering by lineages and sub-lineages as well as isolates were highly diverse and had deep branching (**Figure-3**). The analysis of the clustering rate indicated 17 isolates out of 72 (23.6.0%) were grouped into 4 clusters with each cluster composed of 2 to 7 isolates belonging to the Haarlem sub-lineage. Delhi/CAS and LAM sub-lineages consisting of 1 cluster each composed of 2 isolates.

Table-3: The lineage and sub-lineage placement of the *M. tuberculosis* isolates (n=72)

L=lineage

Lineage	Sub-lineage	Ethiopian isolates (%)
L3 (East-Africa-India)	Delhi/CAS	16 (22.2)
L4 (Euro-American)	Haarlem	15 (20.8)
L4 (Euro-American)	TUR	13 (18.1)
L1 (Indo-oceanic)	EAI	1 (1.4)
L4 (Euro-American)	LAM	6 (8.3)
L4 (Euro-American)	New-1	4 (5.5)
L4 (Euro-American)	URAL	4 (5.5)
-	Not assigned	13 (18.1)
	Total	72 (100)

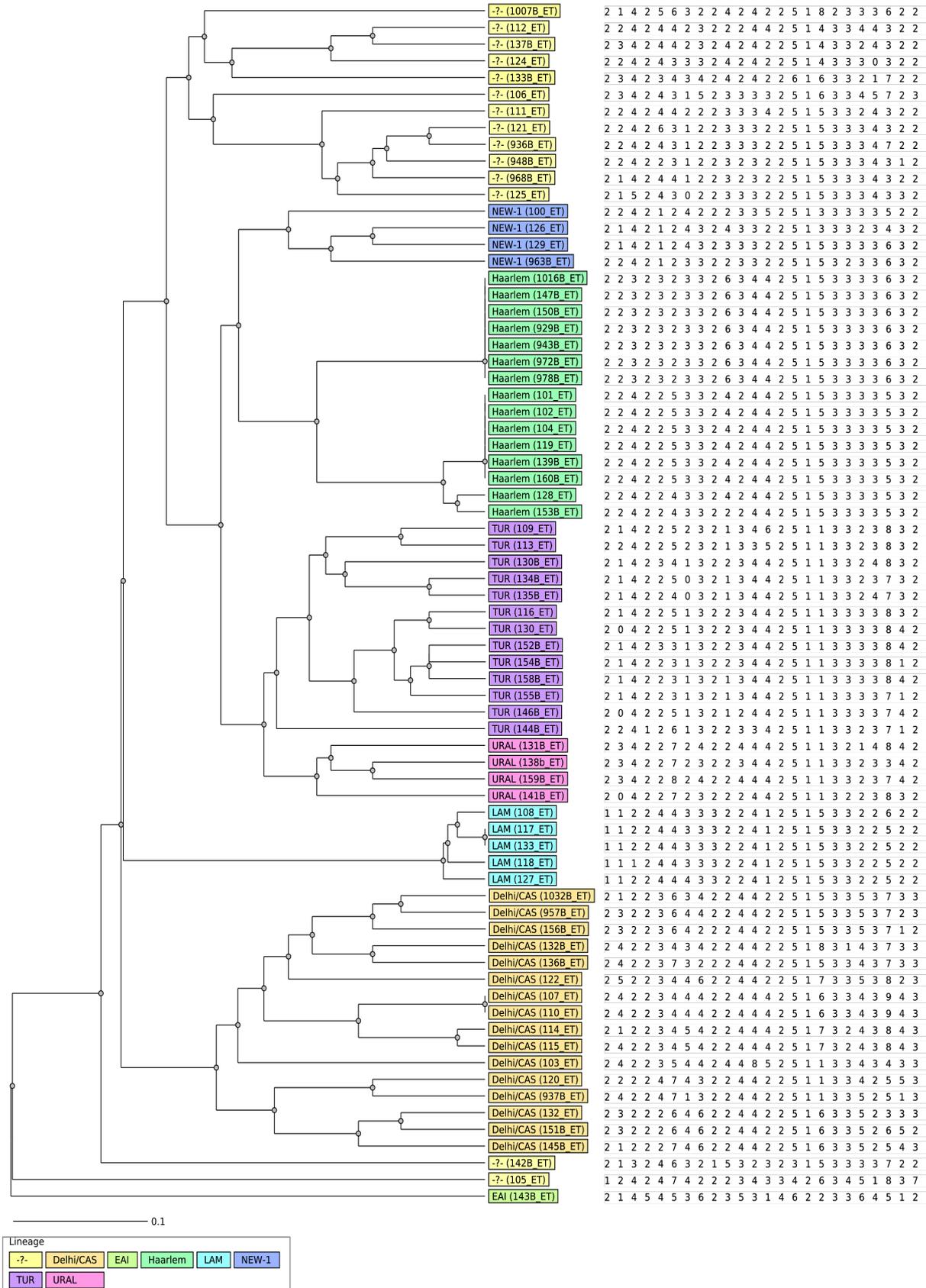


Figure 3: The phylogenetic classification of *M. tuberculosis* isolates isolated from Arsi Zone, during February 2016, and August 2016, Oromia Region, Ethiopia (n=72)

Minimum Spanning Tree and Cluster

The minimum spanning tree (MST) was constructed to identify the clonal complex groups of the isolates within dual locus variants. As shown in **Figure-4**, the lineages are indicated by a different color and, the size of the individual circle shows the number of clusters. All the lineages detected using UPGMA analysis were also detected in MST that further confirm genetic linkage and clustering. Six clonal complexes (CC1-6) consisting of a maximum of 6 and a minimum of 2 isolates with dual locus distance were identified. The largest clonal complex (CC1) (5 isolates) belongs to the TUR sub-lineage

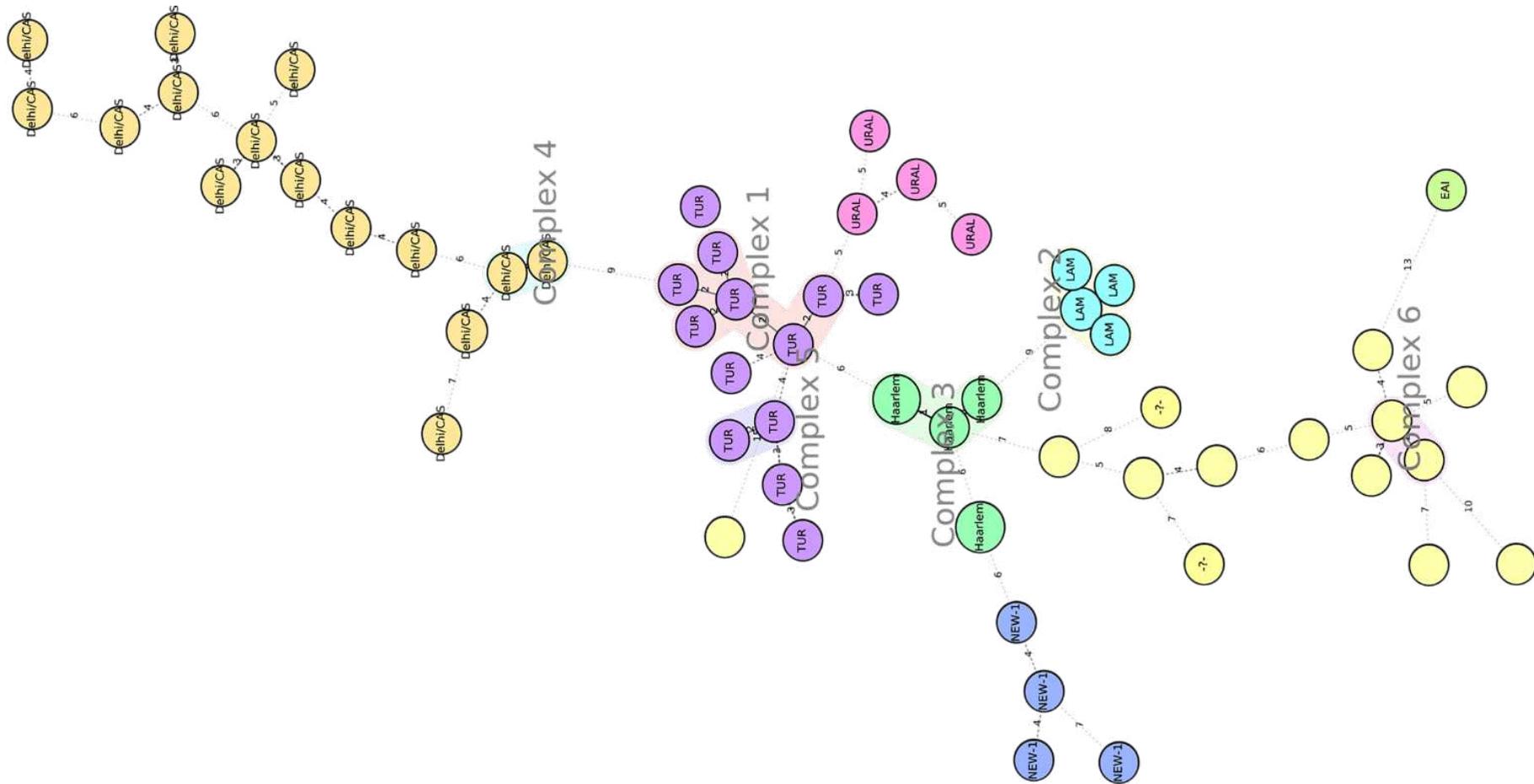


Figure-4: The minimum spanning tree of the *M. tuberculosis* characterized in this study. The number along the broken line connecting individual circle indicates the genetic distance (number of loci differences) between each strain

Discussion

Smear positive PTB is linked to the higher risk of transmission from an infected individual to a health susceptible individual and hence has of great role in TB epidemiology and infection control. In this study, we investigated the prevalence of smear-positive tuberculosis among presumed PTB suspected patients as well as determined the sociodemographic and geographic risks associated with tuberculosis in Arsi Zone. Further, molecular profiling of the isolates was conducted to identify the circulating strains in the area. Genetic typing enhances the detection of major clones associated with various disease outcomes and mapping out of unsuspected outbreaks. Further, characterization is also a foundation for the study of the pathogenesis of the disease that affects virulence, deciphering of the MTBC population structure and enabling the development of new diagnostics, vaccines, or treatments.

Out of 832 presumptive PTB suspected patients, 119 (14.3%) were smear-positive. This result is consistent with the 15.2% reported from Bahir Dar, Northwest Ethiopia [17], 14.2 % reported from Metehara sugar factory hospital; Central Ethiopia [18], and 16.7% reported from Harar TB control center, Eastern Ethiopia [19]. It is worth noting that the relatively higher smear-positive PTB in the current study and similar results mentioned above are associated with the fact that the participants were presumed tuberculosis patients and/or the cases could be referral cases from the district health facility with a higher likelihood of turning smear-positive. The implication however is; those individuals could be highly infectious and need to be closely monitored to enhance the control of tuberculosis.

The higher risk of smear-positive PTB in young age groups (adolescent and young) compared with the old age in this study is concordant to several previous studies [20–22] and with age-stratified tuberculosis estimates published by the Global Burden of Diseases group [23]. This could be related to the fact that these age groups are at their active period of development and have wider social contact outside their household that could increase the chance of acquiring tuberculosis. Furthermore, many comorbidities relevant to tuberculosis emerge or are exacerbated during these ages including risky substance use (including tobacco use), infection with HIV, and mental health conditions. Consequently, the adolescent and young age groups contribute considerably to the ongoing transmission of tuberculosis particularly in the cases of

smear-positive PTB. Provided that these age groups are at higher risk of acquiring tuberculosis necessitates the targeted intervention strategies for effective control of tuberculosis.

After controlling for other factors, the place of residence also was identified as a risk factor in the multivariate analysis. This is contrary to previous studies [24, 25] that identified urban residents as a determinant of the PTB associated with the overcrowded living style in the urban area. On the other hand, the current study is also in agreement with a study from China [26] and a case-control study conducted in Ambo Hospital [27] where being a rural resident is one of the predictors of smear-positive PTB. The discrepancies could be attributed to differences in the target study subject and the lifestyle of the studied population.

Knowledge of the spatial distribution of tuberculosis could augment the control and prevention efforts by identifying high burden areas with low cases detection and weak public health programs. Such identification allows the policymakers to establish priorities for intervention, including TB diagnosis and treatment service to halt the transmission. In this study, significant clustering of smear-positive PTB was identified in 11 (47.8%) districts that cover areas Central to remote Eastern parts of the Arsi zone.

The majority (71.1%) of the isolates belong to the Euro-American lineage which is consistent with the previous reports [10, 28] suggesting the successful and widespread distribution of this lineage in Ethiopia. Further classification of the Euro-American lineage to sub-lineages indicated the deep-branching of the lineage and the high diversity of the strains belonging to this lineage suggesting the higher *M. tuberculosis* genetic repository potential of the area. We also speculate that diversity could also reflect the potential distance between the patients from whom the samples were collected. *M. tuberculosis* Delhi/CAS and Haarlem represent the largest proportion (accounting for 22.2% and 20.8%), respectively. A recent systematic review by Tulu and Ameni, [29] also indicated the high prevalence of the Delhi/CAS and Haarlem sub-lineage among the diverse isolates and similarly, a study by Tessema *et al.*, [30] also identified *M. tuberculosis* Delhi/CAS and Haarlem as 1st and the 3rd common sub-lineage among the isolates included in their study. Though Delhi/CAS is presumed to be geographically localized in Indian and Central Asia, two assumptions were forwarded for the distribution of this sub-lineage in Ethiopia. The currently growing bidirectional economic and social relationship between Ethiopia and India might have increased the transmission dynamics

of these strains from India to Ethiopia. Or East Africa, specifically Ethiopia, is assumed to be the origin of human tuberculosis, thus, strains of *M. tuberculosis* might have migrated to India and central Asia following the “Out of Africa” theory.

Though *M. tuberculosis* TUR sub-lineage is occasionally reported from Ethiopia, possibly associated with few studies conducted using MIRU-VNTR based genotyping, this is the first report that identified the considerable number (18.1%) of TUR sub-lineage which formed large clonal complexes of 7 isolates in the study area. This might be because most genotyping studies in Ethiopia have been conducted by using a spoligotyping method that classifies TUR sublineage as T1, one of the most prevalent sublineages reported in Ethiopia.

Owing to the airborne transmission of *M. tuberculosis*, transmission dynamic estimation using the cluster rate approach would significantly benefit tuberculosis control programs. This would help to identify the factors contributing to the increasing incidence of tuberculosis. Overall, the clustering rate of 26.4% recorded in this study. The clustering rate for isolates in this study is noticeably lower than previous reports in a different part of the country (reviewed, [29]) which could be related to the discrete nature of the patients from whom the isolates were collected. The Haarlem isolates were identified as the major cluster in Ethiopian isolates suggesting that this strain is responsible for the majority of the recent transmission in the study area.

Conclusion

Our study showed higher smear-positive results among PTB suspected patients and remarkable spatial variation across districts of Arsi Zone in smear-positive PTB. This information together the genotypic features could be used as input for the efforts of designing control strategies

Declaration

Ethics approval and consent to participate: We confirm that written informed consent was obtained from all participants of our study and for those whose age were less than 18 years consent were obtained from parent and/or legal guardian . The ethical approval committees of the College of Health, Arsi University (A/U/H/C/87/4016) approved the study protocols. Methods were carried out in accordance with relevant guidelines and regulations.

Consent of publication: Not applicable

Availability of data and materials: The datasets supporting the conclusions of this article are presented within the article (and its additional file)

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

Funding: This work was supported the fund from Arsi University research grant fund (A/CHS/RC/12/15). The funding bodies had no role in the study design, data collection, analysis, and interpretation, or manuscript writing.

Authors' contributions : KT conceived the idea. KT, TKB, SG, AGW and GS contributed to the design of the study, laboratory data collection and analyzed the data. KT and GS drafted the manuscript, and all authors involved in the manuscript writing and approved the manuscript.

Acknowledgment: We would like to express our sincere gratitude to the Arsi University for the financial support and Asella Hospital staff in general and TB clinic staff in particular for their all-round support during data collection

References

1. World Health Organization. Global tuberculosis report 2019.
2. Alene KA, Wagaw ZA, Clements ACA. Mapping tuberculosis prevalence in Ethiopia: Protocol for a geospatial meta-analysis. *BMJ Open*. 2020;10.
3. Alene KA, Viney K, Gray DJ, McBryde ES, Wagnew M, Clements ACA. Mapping tuberculosis treatment outcomes in Ethiopia. *BMC Infect Dis*. 2019;19:474.
4. Alene KA, Clements ACA. Spatial clustering of notified tuberculosis in Ethiopia: A nationwide study. *PLoS One*. 2019;14:e0221027.
5. Dangisso MH, Datiko DG, Lindtjørn B. Identifying geographical heterogeneity of pulmonary tuberculosis in southern Ethiopia: a method to identify clustering for targeted interventions. *Glob Heal Action*. 2020;13:1785737.
6. Lönnroth K, Jaramillo E, Williams BG, Dye C, Raviglione M. Drivers of tuberculosis epidemics: The role of risk factors and social determinants. *Soc Sci Med*. 2009;68:2240-2246.
7. Behr MA, Warren SA, Salamon H, Hopewell PC, Ponce De Leon A, Daley CL, et al. Transmission of *Mycobacterium tuberculosis* from patients smear-negative for acid-fast bacilli. *Lancet*. 1999;353:444–9.
8. Hernández-Garduño E, Cook V, Kunimoto D, Elwood RK, Black WA, FitzGerald JM. Transmission of tuberculosis from smear negative patients: A molecular epidemiology study. *Thorax*. 2004;59:286–90.
9. Trauer JM, Dodd PJ, Gomes MGM, Gomez GB, Houben RMGJ, McBryde ES, et al. The importance of heterogeneity to the epidemiology of tuberculosis. *Clin Infect Dis*. 2019;69(1):159-166.
10. Firdessa R, Berg S, Hailu E, Schelling E, Gumi B, Erenso G, et al. Mycobacterial lineages causing pulmonary and extrapulmonary Tuberculosis, Ethiopia. *Emerg Infect Dis*. 2013;19:460–3.
11. Coscolla M, Gagneux S. Consequences of genomic diversity in mycobacterium tuberculosis. *Semin Immunol*. 2014;16(6): 431–44.
12. Reiling N, Homolka S, Walter K, Brandenburg J, Niwinski L, Ernst M, et al. Clade-specific virulence patterns of *Mycobacterium tuberculosis* complex strains in human primary macrophages and aerogenically infected mice. *MBio*. 2013;4:e00250-13.
13. Forbes BA, Hall GS, Miller MB, Novak SM, Rowlinson MC, Salfinger M, et al. Practice guidelines for clinical microbiology laboratories: Mycobacteria. *Clin Microbiol Rev*. 2018;31:1–66.
14. Yam WC, Tam CM, Leung CC, Tong HL, Chan KH, Leung ETY, et al. Direct detection of rifampin-resistant *Mycobacterium tuberculosis* in respiratory specimens by PCR-DNA sequencing. *J Clin Microbiol*. 2004;42:4438–43.
15. Supply P, Allix C, Lesjean S, Cardoso-Oelemann M, Rüsch-Gerdes S, Willery E, et al. Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of *Mycobacterium tuberculosis*. *J Clin Microbiol*. 2006;44:4498–510.
16. Allix-Béguet C, Harmsen D, Weniger T, Supply P, Niemann S. Evaluation and strategy for use of MIRU-VNTRplus, a multifunctional database for online analysis of genotyping data and phylogenetic identification of *Mycobacterium tuberculosis* complex isolates. *J Clin Microbiol*. 2008;46:2692–9.
17. Andualem Asemahagn M. Determinants of Sputum Smear Positivity among Tuberculosis Suspected Patients in Bahir Dar City, Northwest Ethiopia. *Ann Med Heal Sci Res*. 2018;8:111–7.
18. Yohanes A, Abera S, Ali S. Smear positive pulmonary tuberculosis among suspected

patients attending metehara sugar factory hospital; eastern Ethiopia. *Afr Heal Sci.* 2012;12:325–30.

19. Hylemariam M, Daniel G, Yaregal A, Diriba G, Eskedar T, Berhanu S. Prevalence of Smear positive Pulmonary Tuberculosis (PTB) and Associated Factors among PTB Suspects Attending Harar Tuberculosis Control Center, Harar, Ethiopia. *J Med Lab Diagn.* 2018;9:1–5.

20. Snow KJ, Sismanidis C, Denholm J, Sawyer SM, Graham SM. The incidence of tuberculosis among adolescents and young adults: A global estimate. *Eur Respir J.* 2018;51:1702352.

21. Marais BJ, Gie RP, Schaaf HS, Hesselning AC, Obihara CC, Nelson LJ, et al. The clinical epidemiology of childhood pulmonary tuberculosis: A critical review of literature from the pre-chemotherapy era. *Int J Tuberc Lung Dis.* 2004;8 (3): 278-285.

22. Morabia A. Snippets from the past: Cohort analysis of disease rates-another piece in a seemingly still incomplete puzzle. *Am J Epidemiol.* 2014;180:189–96.

23. Murray CJL, Ortblad KF, Guinovart C, Lim SS, Wolock TM, Roberts DA, et al. Global, regional, and national incidence and mortality for HIV, tuberculosis, and malaria during 1990-2013: A systematic analysis for the Global Burden of Disease Study 2013. *Lancet.* 2014;384:1005–70.

24. Abebe DS, Bjune G, Ameni G, Biffa D, Abebe F. Prevalence of pulmonary tuberculosis and associated risk factors in Eastern Ethiopian prisons. *Int J Tuberc Lung Dis.* 2011;15:668–73.

25. Berju A, Haile B, Nigatu S, Mengistu A, Birhan G. Smear-Positive Tuberculosis Prevalence and Associated Factors among Pregnant Women Attending Antenatal Care in North Gondar Zone Hospitals, Ethiopia. *Int J Microbiol.* 2019;17:100–8.

26. Chen W, Shu W, Wang M, Hou Y, Xia Y, Xu W, et al. Pulmonary Tuberculosis Incidence and Risk Factors in Rural Areas of China: A Cohort Study. *PLoS One.* 2013;8:e58171.

27. Ephrem T, Mengiste B, Mesfin F, Godana W. Determinants of active pulmonary tuberculosis in Ambo Hospital, West Ethiopia. *Afr J Prm Heal Care Fam Med.* 2015;7:1–8.

28. Tadesse M, Abebe G, Bekele A, Bezabih M, de Rijk P, Meehan CJ, et al. The predominance of Ethiopian specific *Mycobacterium tuberculosis* families and minimal contribution of *Mycobacterium bovis* in tuberculous lymphadenitis patients in Southwest Ethiopia. *Infect Genet Evol.* 2017;15:251–9.

29. Tulu B, Ameni G. Spoligotyping based genetic diversity of *Mycobacterium tuberculosis* in Ethiopia: A systematic review. *BMC Infect Dis.* 2018;18.

30. Tessema B, Beer J, Merker M, Emmrich F, Sack U, Rodloff AC, et al. Molecular epidemiology and transmission dynamics of *Mycobacterium tuberculosis* in Northwest Ethiopia: New phylogenetic lineages found in Northwest Ethiopia. *BMC Infect Dis.* 2013;13.

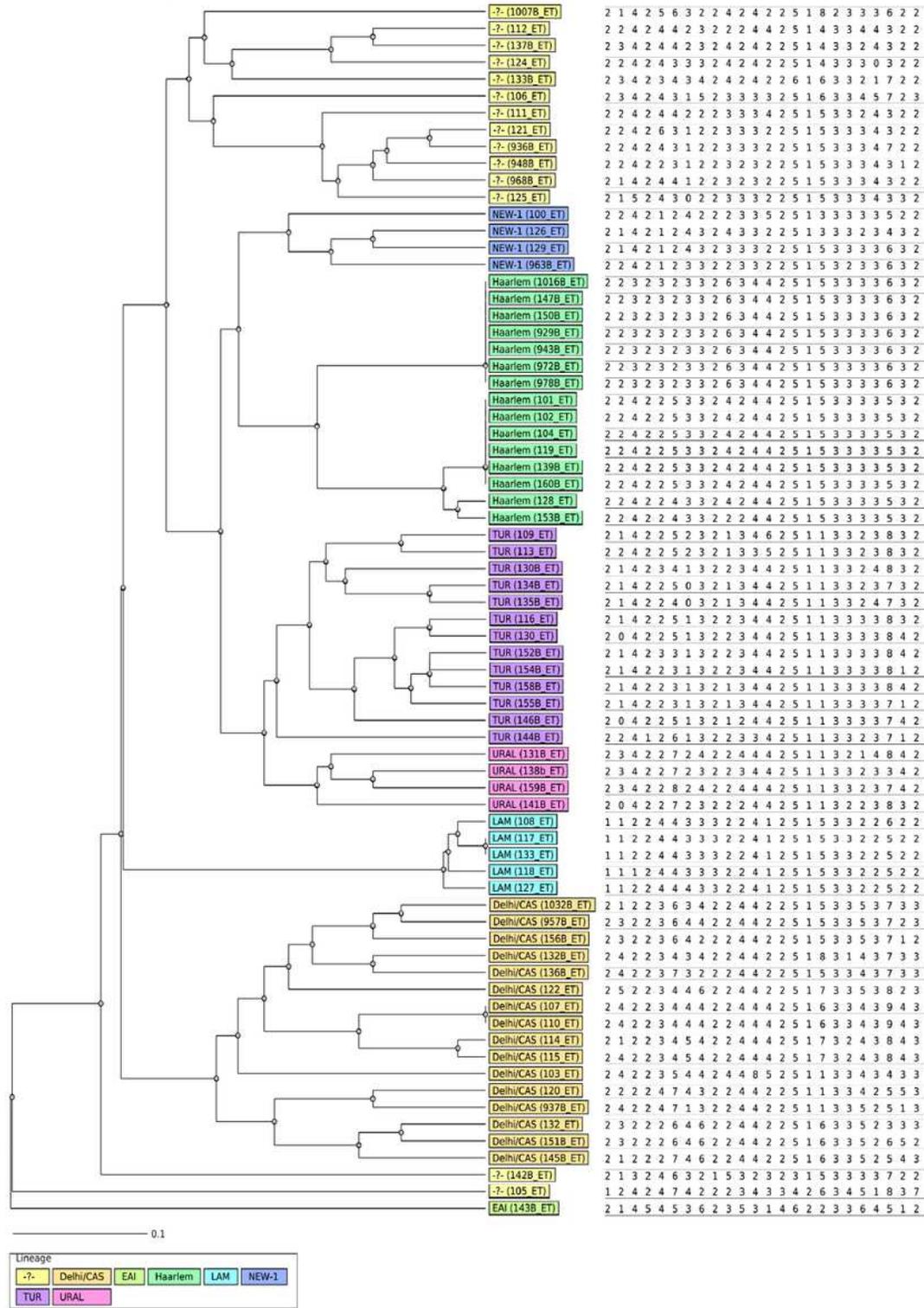


Figure 2

The phylogenetic classification of *M. tuberculosis* isolates isolated from Arsi Zone, during February 2016, and August 2016, Oromia Region, Ethiopia (n=72)

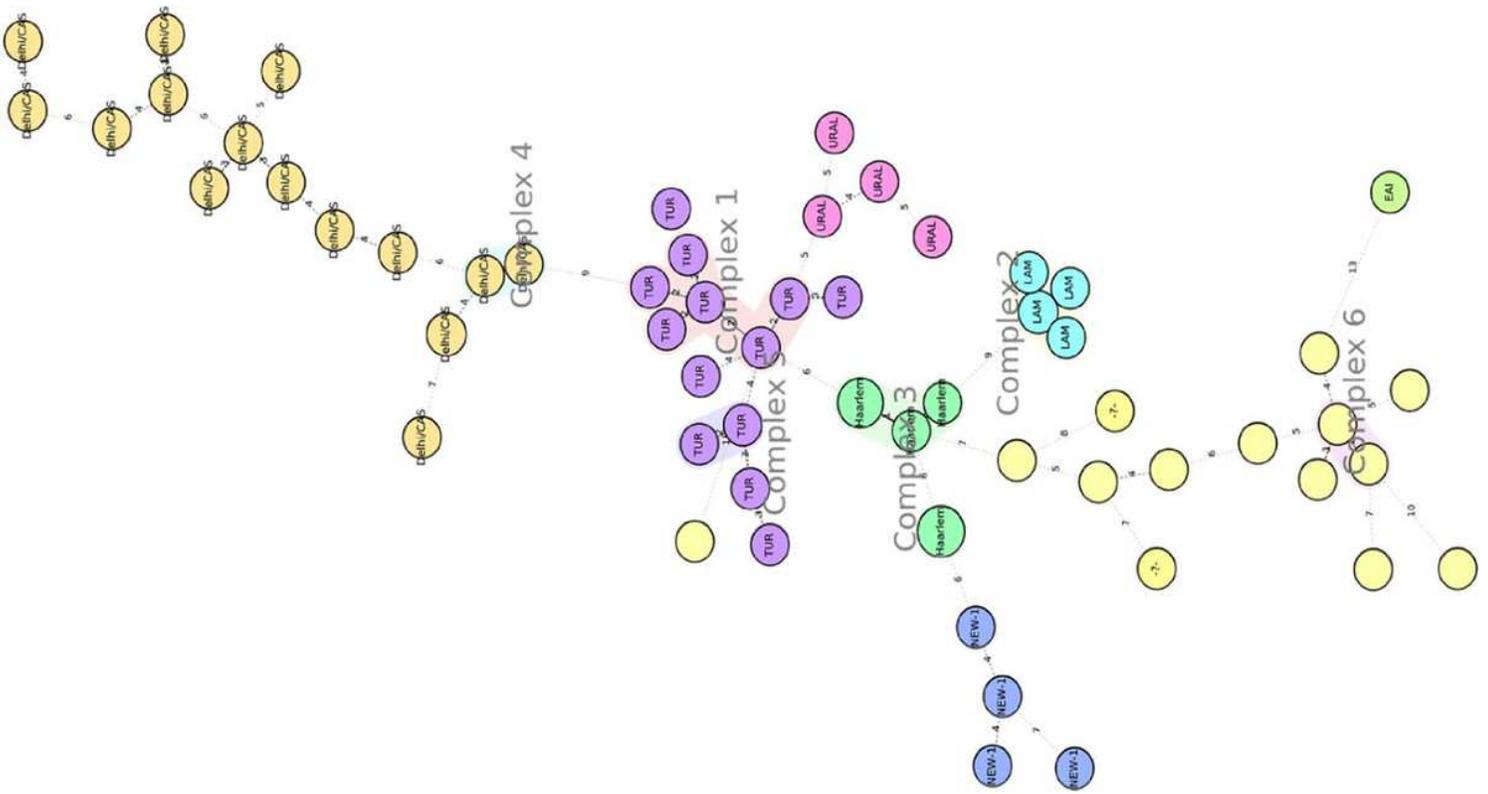


Figure 3

The minimum spanning tree of the *M. tuberculosis* characterized in this study. The number along the broken line connecting individual circle indicates the genetic distance (number of loci differences) between each strain

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryfigureandTable.docx](#)
- [SupplementaryfigureandTable.docx](#)
- [SupplementaryfigureandTable.docx](#)