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Review

Molecular epidemiology of *M. tuberculosis* in Ethiopia: A systematic review and meta-analysis

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ABSTRACT

The molecular epidemiology of *Mycobacterium tuberculosis* (*M. tuberculosis*, Mtb) is poorly documented in Ethiopia. The data that exists has not yet been collected in an overview metadata form. Thus, this review summarizes available literature on the genomic diversity, geospatial distribution and transmission patterns of *Mtb* lineages (L) and sublineages in Ethiopia. Spoligotyping and Mycobacterial Interspersed Repetitive Units-Variable Number Tandem Repeats (MIRU-VNTR) based articles were identified from MEDLINE via PubMed and Scopus. The last date of article search was done on 12th February 2019. Articles were selected following the PRISMA flow diagram. The proportion of (sub)lineages was summarized at national level and further disaggregated by region. Clustering and recent transmission index (RTI) were determined using metan command and random effect meta-analysis model. The meta-analysis was computed using Stata 14 (Stata Corp. College Station, TX, USA). Among 4371 clinical isolates, 99.5% were *Mtb* and 0.5% were *M. bovis*. Proportionally, L4, L3, L1 and L7 made up 62.3%, 21.7%, 7.9% and 3.4% of the total isolates, respectively. Among sublineages, L4.2. ETH/SIT149, L4.10/SIT53, L3. ETH1/SIT25 and L4.6/SIT37 were the leading clustered isolates accounting for 14.4%, 9.7%, 7.2% and 5.5%, respectively. Based on MIRU-VNTR, the rate of clustering was 41% and the secondary case rate from a single source case was estimated at 29%. Clustering and recent transmission index was higher in eastern and southwestern Ethiopia compared with the northwestern part of the country. High level of genetic diversity with a high rate of clustering was noted which collectively mirrored the phenomena of micro-epidemics and super-spreading. The largest set of clustered strains deserves special attention and further characterization using whole genome sequencing (WGS) to better understand the evolution, genomic diversity and transmission dynamics of Mtb.

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List of abbreviations

ANI	average nucleotide identity
BCG	bacille Calmette-Guerin
CAS	Central Asia
Dddh	digital DNA–DNA hybridization
DR	Direct repeats
DS-TB	drug-sensitive TB
EA	Euro-American
EAI	EastAfrican Indian
EAS	East Asia
EPTB	extra-pulmonary tuberculosis
ES	study estimate
ETH	Ethiopia
IPT	Isoniazid prophylaxis treatment
JBI	The Joanna Briggs Institute
LAM	Latin American and Mediterranean
M.tb	*M. tuberculosis*
M/F	Male/Female
MDR-TB	multidrug resistant TB
MIRU-VNTR	Mycobacterial Interspersed Repetitive Units-Variable Number Tandem Repeats
MIT	MIRU-VNTR international type
MTBC	*Mycobacterium tuberculosis* complex
NGS	next-generation sequencing
NTM	nontuberculosis Mycobacteria
PGRS	polymorphic G + C rich sequences
PTB	Pulmonary tuberculosis
PTB$^-$	sputum smear negative pulmonary tuberculosis
PTB$^+$	sputum smear positive pulmonary tuberculosis;
RTI	Recent transmission index
RR	rifampicin resistance
S	Sicily
SIT	Spoligotyping international type
SNNP	Southern nations, nationalities and people's
SNPs	single nucleotide polymorphisms
STROME-**ID**	Strengthening the Reporting of Molecular Epidemiology for Infectious Diseases
T	Tuscany
TB	Tuberculosis
TBLN	Tuberculous lymphadenitis
WGS	whole genome sequence
WHO	World Health Organization

1. Introduction

Tuberculosis (TB) is a complex and chronic infectious disease of the lung, the lymph node and other parts of the body. According to the 2018 global TB report, there were 10 million infections and 1.6 million deaths due to TB in 2017 [1].

In Ethiopia, the incidence of TB was 172/100,000 and TB/HIV co-infection rate was 12% [1]. However, rates as high as 26% TB/HIV co-infection prevalence had previously been reported by other studies [2,3]. The 117,705 TB cases notified in 2017 represented 68% of the estimated incidence [1]. Reports showed that, 32% and 30% of TB cases are extra-pulmonary TB (EPTB) and sputum smear negative pulmonary TB (PTB$^-$), respectively [4–7]. The remaining 38% are sputum smear-positive pulmonary TB (PTB$^+$) which are likely responsible for most of the transmission [8]. The multidrug resistance/rifampicin resistance (MDR/RR) rate was 2.18% and 21% among new and previously treated TB cases, respectively [9]. Moreover, the treatment success rate was 90% for new TB cases [1] and 59.2% for MDR/RR TB cases [10].

Previous host genomic [11-16], sociodemographic [17] and pathogen genomic [18,19] studies have attempted to define the determinants leading to PTB, EPTB and latent TB in different populations. However, results across different studies are heterogeneous and in conflict with each other.

Species of TB causing *Mycobacteria* were grouped as *Mycobacterium tuberculosis complex* (MTBC) [20] to delineate them from non-tuberculosis Mycobacteria (NTM), *M. leprae*, *M. ulcerans* and environmental *Mycobacteria* [21–24]. However, Riojas et al. (2017) using next-generation sequencing (NGS), digital DNA–DNA hybridization (dDDH), and average nucleotide identity (ANI) data detected high degree of identity (dDDH: 91.2–99.2%, ANI: 99.21–99.92%) among the species of MTBC. Based on these data, these authors challenged the species nomenclature of the MTBC complex and recommend infrasubspecific designations using the term “variant” (var.) such as *M. tuberculosis* var. tuberculosis, *M. tuberculosis* var. africanum [25].

Mtb genotypes are geographically structured in to seven major lineages and several sublineages [26,27]. Lineage and sublineage classification is based on the work of Brudy et al. [26], Gagneux and Small [27] and Coll et al. [28]. The seven major lineages includes lineage 1 (L1: East African Indian/EAI/or Indo-Oceanic), L2 (East Asia, EAS, Beijing), L3 (Central Asia, CAS), L4 (Euro-American, EA), L5 and L6 (*M. africanum* I and II) and L7. Sublineages are spoligotyping families under each lineage.

Based on best available evidence, East Africa in particular Ethiopia, is the most likely evolutionary origin of the recent most common ancestor of Mtb [29] and *Homo sapiens* [30]. As such, Ethiopia hosts diverse specialist and generalist types of Mtb strains [31] that have established sympatric and allopatric association with their hosts [32,33]. These evolutionary trajectories together with changing social and economic determinants, are probably responsible for the current unique epidemiology of TB in Ethiopia (including a high proportion of lymph node tuberculosis, 32% of total vs 38% PTB$^+$, and 30% PTB$^-$).

The complete genome of Mtb is nearly 4.4 mega base pairs (Mbp) long and is subdivided into RNA coding sequences, repetitive DNA sequences, direct repeats (DR), PE/PPE family members, polymorphic G + C rich sequences (PGRS) and protein coding sequences [34]. Strains have over 99% genomic similarity; on average differing by about 1200 single nucleotide polymorphisms (SNP) [35]. Such clonality was maintained through absence of horizontal gene transfer, selective sweeps, purifying selection, background selection, and transmission bottlenecks [31]. Mtb molecular typing relies on the above-mentioned genomic regions and the principles of each typing techniques are reviewed elsewhere [36]. Briefly, spoligotyping is based on DR regions which contain 43 sets of repetitive spacer sequences interspersed with 34–41 base pair (bp) long nonrepetitive sequences [37]. The MIRU-VNTR with 24 loci is used for classification of isolates into (sub) lineages and is currently the minimum recommended technique for the analysis of transmission pattern. However, for high resolution molecular epidemiology and evolutionary analysis, working with whole genome SNPs is superior [38–43].

Contact screening has served as a tool to both study and control TB transmission control. A simplified TB transmission dynamics model contains five groups: susceptible, latently infected, infectious, non-infectious and recovered cases [44]. Over time, an individual might move from one state to another through spontaneous cure and relapse [44]. The “stone-in-the-pond” principle of contact tracing is the gold standard non-molecular method for the identification of the transmission chain in TB infection [45]. A symptom-based contact screening of 15, 527 people whose source cases were PTB$^+$ was done in Amhara and Oromia region between 2013 and 2014. Of these, 6.1% had presumptive TB. All forms of TB and PTB$^+$ were diagnosed in 2.5% and 0.76% of contacts, respectively [46]. A similar study also screened 272,441 close contacts of 47, 021 index cases. Of these, 5.1% and 0.8% had presumptive and

active TB, respectively [47]. Another study compared the prevalence of TB among contacts of MDR-TB and drug-sensitive (DS) PTB^{+} index cases. The study screened 331 MDR-TB and 353 DS-TB contacts where the prevalence of TB among contacts was 2.7% and 4.0%, respectively [48].

Contact screening-based transmission analysis does not always link the true transmission chain [49,50]. Thus, currently, molecular typing is used to determine the chain and timing (cluster age) of transmission [43]. In Ethiopia, MIRU-VNTR and spoligotyping have been used for molecular typing and transmission analysis. Recently, a systematic review of spoligotyping based Mtb genetic diversity was reported [51]. However, this study excluded MIRU-VNTR based articles. Moreover, nationally representative pooled transmission dynamics data were not provided. Thus, this review aimed to summarize existing spoligotyping and MIRU-VNTR based articles: (1) to determine the diversity and phylogeography of (sub) lineages, (2) to identify the dominant spoligotyping international types (SIT) so as to bring them into sharper focus for further study and (3) to characterize the transmission pattern.

2. Methods

2.1. Eligibility criteria

For the analysis of the genomic diversity, phylogeography and transmission patterns, both spoligotyping and MIRU-VNTR based articles were included. However, for clustering and RTI analysis, only MIRU-VNTR based articles were considered. Articles from Ethiopia that characterized Mtb (sub) lineages published in English language irrespective of publication year, demography, clinical and TB types were included.

2.2. Information sources and search strategy

MEDLINE through PubMed and Scopus were the source of articles and last date of article search was 12th February 2019. Additionally, the reference list of some reviews was used to retrieve further literature. The search was done using medical subject headings (MeSH terms) and Boolean operators such as 'molecular typing', or 'molecular epidemiology', and '*M. tuberculosis*', and 'Ethiopia' (supplementary file1, S1).

2.3. Study selection

All of the identified articles were exported to EndNote library. After removing the duplicate, screening was done by title followed by reading the abstract and then by reviewing the full work. Articles that did not fulfill the inclusion criteria were excluded. Articles were independently assessed for inclusion by two authors of this paper (DM, EN). Disagreements regarding the inclusion or exclusion of articles were resolved by discussion among authors.

2.4. Data collection process and data items

Data from the included articles were extracted independently by two authors using data extraction sheet. Disagreements on the extracted data were resolved by discussion. The extraction included: author and year of publication, study period, molecular techniques, sample size,

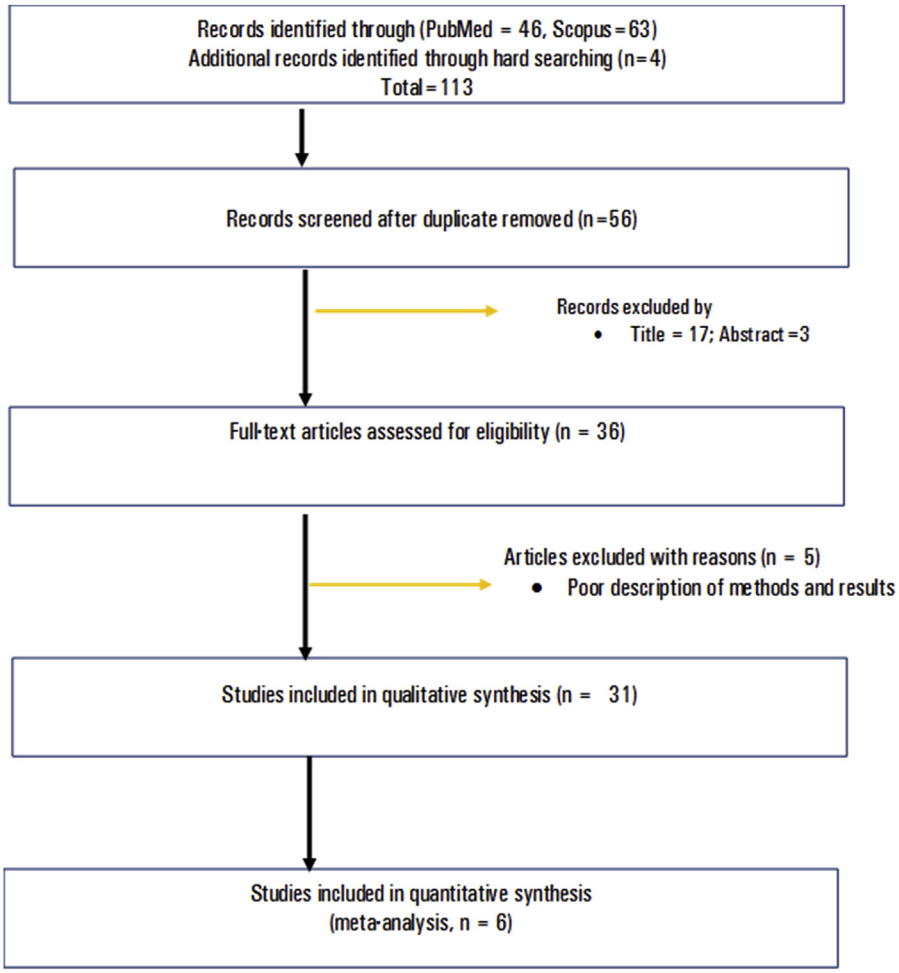

Fig. 1. Flow diagram showing selection process of articles, 2005–2018.

type and history of TB, sex and age classification, HIV status, study area, types and number of (sub) lineage, SIT, cluster size, number of isolates within the cluster and non-clustered isolates.

2.5. Risk of bias in individual studies

Methodological quality of included articles was appraised in duplicate (by DM & AD) using customized check lists of Strengthening the Reporting of Molecular Epidemiology for Infectious Diseases (STROME-ID) [52] and the Joanna Briggs Institute (JBI) [53]. The customized tool has ten domains and each domain scores either one or zero point. Discordance on rating was resolved by the third author (FB). The quality of each article was rated and judged.

2.6. Summary measures and synthesis of results

The extracted data were summarized using frequency and percentage. The national Mtb population genomic structure was divided into (sub) lineages and SIT and then summarized using tables. The geographic distribution of sub (lineages) was mapped using ArcGIS 10.3 (ArcGIS Desktop, ESRI 2011. Redlands, Canada). The spatial data used for the maps were taken from Map library which is a public domain that can be accessed at www.maplibrary.org. Clustering and RTI were analyzed using Stata 14 (Stata Corp. College Station, TX, USA).

Tuberculosis cases were said to be clustered when strains isolated from different patients showed similar genotyping patterns [54]. Recent transmission index is the average number of secondary cases of the disease from a single primary/source/case [55]. The RTI from individual articles was calculated using RTI (n-1) method described elsewhere [56]; where RTI = (nc-c)/N, 'N' is the total number of cases, 'nc' is the number of isolates within the cluster and 'c' is the number of clusters or source cases.

Approximating likelihood approach was followed. In addition, to preserve the deviations, Freeman-Tukey double arcsine rooted transformation was done [57]. To estimate the transformed pooled prevalence, Dersimonian and Laird method was used [58]. Together, using the metan command in Stata, study estimate (ES) as prevalence was computed using Freeman-Tukey double arcsine root transformation with 95% confidence interval with the approach of Dersimonian and Laird method.

2.7. Risk of bias across studies

Statistical heterogeneity estimate among the articles was judged using I^2 statistic and P-value of above and below 0.05. The I^2 value of < 25%, 25–50% and > 50% was taken as low, moderate and high degree of heterogeneity, respectively [59]. Moreover, publication bias was assessed using funnel plot asymmetry. To assess the influence of an individual study on pooled prevalence, sensitivity analysis was performed.

Table 1
Profile of included articles, Ethiopia, 2005–2018.

Author, year	Typing	Study Period	Region	Sample Size (PTB/TBLN)	Types (N/R)	Sex (M/F)	Age*	HIV (Y/N/U)
Agonafir, 2010 [60]	SIT	2005–2006	Ethiopia	114/0	48/66	70/44	5/82/20	ND
Ali 2016 [61]	SIT + 24 MIT	2013	O/SNNP/H/DD	127/0	105/4/	64/45	0/99/10	ND
Ameni, 2013 [62]	SIT	ND	Fiche	141/0	ND	74/72	ND	ND
Bedewi, 2017 [63]	SIT	2012–2013	Woliso/Atat	281/0	271/10	151/130	0/171/110	ND
Belay, 2014 [64]	SIT	2009–2010	Afar	105/0	81/24	69/36	0/105	37/58/10
Biadglegne 2015 [65]	SIT + 24 MIT	2012	Amhara	0/226	183/11	73/121	21/156/27	ND
Chemeda 2018 [66]	SIT	2014	AA	143/0	ND	55/88	0/125/18	143/0/0
Debebe 2014 [67]	SIT	2010–2011	Bahir Dar	118/0	116/2	66/52	6/93/19	12/106/0
Deribew 2012 [68]	SIT	2009	Jimma	17/0	5//4	7//10	NI	ND
Diriba 2013 [69]	SIT	2009–2012	Ethiopia	183/0	3/180	100/83	0/169/14	ND
Disassa 2015 [70]	SIT	2012–2013	BG	33/0	ND	31//22	0/48/5	ND
Firdessa 2013 [71]	SIT + 24 MIT, SNP	2006–2010	Ethiopia	622/328	N/N	331/246//138/168	128/355/87//120/148/21	ND
Garedew 2013 [72]	SIT	2010–2011	Debre Birhan	0/98	89/9	ND	16//66//16	ND
Garedew 2013 [73]	SIT	2010–2011	Debre Birhan	99/0	80/19	43/56	0/77/22	ND
Gebrezgabiher 2015 [74]	SIT	2012–2013	Gedeo	76/21	ND	ND	ND	ND
Getahun 2015 [75]	SIT	2010–2011	Ethiopia	96/0	ND	51/45	0/70/26	ND
Gumi 2012 [76]	SIT	2008–2010	Guji & Liben	164/9	ND	ND	ND	ND
Korma 2015 [77]	SIT	2012–2013	AA	53/116	167/33	94/106	21/161/18	62/123/15
Maru 2015 [78]	SIT	2012–2013	Dessie	144/0	128/16	80/64	10/119/15	25/119/0
Mekonnen 2018 [79]	SIT + 24 MIT	2016–2017	O/S/H/DD	160/0	114/46	128/32	16/124/20	17/143/0
Mihret 2012 [80]	SIT	2009–2010	AA	192/0	192/0	109/83	ND	26/136/30
Molina-Moya 2018 [81]	SIT	ND	SNNP	91/0	ND	ND	ND	ND
Nuru 2015 [82]	SIT	2012–2014	Bahir Dar	54/114	142/26	80/88	0/134/34	ND
Nuru 2017 [83]	SIT	2012–2014	Bahir Dar	0/70	ND	52/18	0/41/29	ND
Tadesse 2017 [84]	SIT + 15MIT, SNP	2013–2015	Jimma	0/304	ND	143/161	33/246/25	24/232/48
Tessema 2013 [85]	SIT + 24 MIT	2009	Amhara	260/0	201/43	142/102	7/203/34	62/182/0
Tilahun 2018 [86]	SIT	2014–2015	Ambo	105/0	105/0	43/43	0/90/15	8/42/36
Workalemahu 2013 [87]	SIT	2011	Jimma	121/0	121/0	69/52	121/0/0	15/106
Yimer 2013 [88,89]	SIT + 24MIT	2008–2010	Amhara	240/0	240/0	131/109	0/240/0	51/180
Zewdie 2016 [90]	SIT	2013	AA	0/206	62/12	94/112	0/119/13	ND
	Ratio			3739/1492	2453/505	2488/2188	504/3241/598	482/1427/139
	%			71.5/28.5	83/17	53/47	11.6/74.6/13.8	23.5/69.7/6.8

Age*(< 15/15–44/≥45); **SIT:** Spoligotyping international type; **MIT:** MIRU-VNTR international type; **SNNP:** Southern nations, nationalities and people's; **BG:** Benishangul Gumuz; **O/SNNP/H/DD**: Oromia/SNNP/Hareri/Dire Dawa; **O/S/H/DD:** Oromia/Somali/Hareri/Dire Dawa; **M/F:** Male/Female, **PTB:** Pulmonary tuberculosis; **TBLN:** tuberculous lymphadenitis; **ND:** No data found; **AA**: Addis Ababa; **N/R**: New/retreated; **P/N/U: Po**sitive/Negative/Unknown.

3. Results

3.1. Study selection

A total of 113 articles were retrieved from MEDLINE, Scopus and reference lists of reviews. After removing 57 duplicate articles; 56 articles were screened by title, abstract and full work as per the inclusion criteria. Finally, 31 articles fulfilled the eligibility criteria and were enrolled in the review. The article selection was carried out following the PRISMA flow diagram (Fig. 1).

3.2. Study characteristics

Of the total thirty-one articles, twenty-four used SIT and the rest seven used combined SIT and MIRU-VNTR techniques. The study period lay between 2005 and 2018 (14 years). Twenty articles focused on PTB, six articles on TB lymphadenitis (TBLN) cases and the rest five included both PTB and TBLN cases (with a few non TBLN extra-pulmonary TB cases). Based on the available data presented in Table 1, 71.5%, 83%, 53%, 74.6% and 23.5% were PTB, new TB cases, male, within the age range of 15–44 years and HIV positive, respectively (see Table 1).

3.3. Risk of bias within studies

The articles were appraised based on their molecular methods and associated outcomes. Thus, all the 31 articles obtained a score of five and above out of 10 points. Hence, overall risk of bias judgment is considered low and the articles were judged as of good quality (Table 2).

3.4. M. tuberculosis genomic diversity in Ethiopia

From the total 4371 clinical isolates (S2A), 99.5% were *M. tuberculosis sensu stricto,* and *M. bovis* made up the remaining 0.5%. The L4/EA, L3/CAS, L1/EAI and L7 shared 62.3%, 21.7%, 7.9% and 3.4%, respectively and the rest 3.6% of isolates were unclassified at lineage level (Table 3).

Extending down to sublineages, Tuscany (T) constituted 42.8% followed by Delhi/CAS 16.5% and Haarlem (H) 10.2%. Of specific genotypes and SITs, L4.2. ETH1/SIT149, L4.10/SIT53, L3. ETH1/SIT25 and L4.6/SIT37 were the leading clustered isolates accounting for 14.6%, 9.7%,7.2% and 5.5% of the total isolates, respectively (Tables 3 and S2A). Taken together, the data from S2A and Table 3 support the conclusion that, population genetic structure of Mtb in Ethiopia is very diverse.

3.5. Ethiopian M. tuberculosis phylogeography

After removing low frequency isolates (L2 and *M. bovis*) and regionally unclassified study [75], 4222 isolates were mapped to their place of isolation (S2B). Some studies included multi-regional data. Thus, the area was categorized as Amhara, Afar, Southern Nations Nationalities and Peoples (SNNP), Benishangul Gumuz (BG), central Ethiopia (which included Addis Ababa, Fiche, Woliso, Atat, Butajira), south eastern Ethiopia which included (Dire Dawa, Somali, Harari, Guji, Liben, Filtu) and, other Oromia. Figs. 2 and S2B shows some regional variation in terms of both (sub) lineages. For instance, L1 was proportionally higher in Afar and L3 and L7 were relatively more prevalent in Amhara. L4 was relatively higher in Oromia (77%), SNNP (80%), central Ethiopia (67%) and SEE (72%). Likewise, the L4 sublineage of H is slightly higher in Amhara region; L4 sublineage of S is more frequent in Oromia. On the other hand, the L4 sublineage of T which is the largest family of isolates is lower in Benishangul Gumuz (Fig. 2 A and B, S2B).

3.6. Transmission patterns

Out of the total of 4371 clinical isolates captured for transmission pattern analysis , 3291 (75.3%) isolates were grouped in to 146 different SIT clusters. The maximum cluster size contained 638 isolates. A total of 113 non-clustered SIT isolates were reported. Thus, 259 different SITs were identified in the present review. The remaining 967 isolates were non-SIT and were found as clustered or unique (S2A). To inspect for possible presence of super-spreaders who are infectious cases that produce more secondary cases than similar other index cases; we analyzed the data as per Ypma et al. [8]. Hence, similar topology was found suggesting the presence of super-spreader events (Fig. 3).

Six articles contained MIRU-VNTR based clustering and RTI data [56,61,79,84,85,89]. Their data collection period span six years (2013–2018). Clinical type and region wise sub-group meta-analysis was performed. The overall pooled clustering rate was 41% (95%CI:32–50%) (Fig. 4). The proportion of pooled clustering rate was 46% in eastern and southwestern Ethiopia and 36% in the northwestern part of the country (Fig. 4A). Fig. 4B depicts the rate of clustering among PTB and TBLN cases at 42% (95%CI: 28–56) and 39% (95%CI: 31–47), respectively. S2C depicts the stepwise cluster and RTI standard error rooted transformations (pooled prevalence) for the six included articles.

The nationwide pooled RTI was 29% (95%CI: 21–37) (Fig. 5). Further, TB clinical type and region wise sub-group meta-analysis was also performed. Hence, the pooled RTI was higher in eastern and southwestern Ethiopia, 34%, than in northwestern part of the country , 25% (Fig. 5A). As shown in Fig. 5B, the pooled RTI was also higher among PTB (30%) than among TBLN (26%) cases.

3.7. Risk of bias across studies

The P-value and I^2 statistics showed heterogeneity across studies. To sort out the cause, publication bias was assessed. Part A and B of S3 shows the funnel plot which demonstrate publication bias. Sensitivity analysis also showed no influence of individual study on pooled estimate (S3C). Thus, methodological (demography, study setting, study population) and clinical (TB types, history, co-morbidity) heterogeneity might be responsible for heterogeneity across studies. Moreover, the number of included articles were small and this might also contribute to the heterogeneity. This collectively mirrored the low validity of the clustering and RTI estimates. Thus, readers are advised to interpret the clustering and RTI estimates with heterogeneity in mind.

Table 2
Risk of bias appraisal summary of included articles, Ethiopia, 2005–2018.

Author, Year	OQS	Author, Year	OQS
Agonafir, 2010 [60]	7	Gumi, 2012-PTB [76]	6
Ali, 2016 [61]	6	Korma, 2015 [77]	8
Ameni, 2013 [62]	6	Maru, 2015 [78]	6
Bedewi, 2017 [63]	6	Mekonnen, 2018 [79]	10
Belay, 2014 [64]	6	Mihret, 2012 [80]	6
Chemeda, 2018 [66]	5	Molina-Moya, 2017 [81]	5
Debebe, 2013 [67]	5	Nuru, 2015 [82]	6
Deribew, 2012 [68]	5	Nuru, 2017 [83]	6
Diriba, 2013 [69]	6	Tadesse, 2017 [84]	7
Disassa, 2015 [70]	5	Tessema 2013 [85]	7
Biadglegne, 2015 [65]	7	Tilahun, 2018 [86]	7
Firdessa, 2013 [71]	8	Workalemahu, 2013 [87]	5
Garedew, 2013 [72]	5	Yimer, 2013 [88]	8
Garedew, 2013 [73]	5	Yimer, 2015 [89]	8
Gebrezgabiher, 2015 [74]	5	Zewdie, 2016 [90]	6
Getahun, 2015 [75]	6		

OQS: Overall quality score.

Table 3
Genomic diversity of *Mycobacterium tuberculosis* in Ethiopia, 2005–2018 (N = 4371).

Lineages, %	Sublineages, Number (%)		Sublineage prevalence among	
			PTB, n (%)	TBLN, n (%)
L1/EAI,7.9	EAI	73 (1.7)	43 (59)	30 (41)
	MANU	202 (4.6)	177 (87.6)	25 (12.4)
	Family 32/33/34/36	69 (1.6)	64 (92.8)	5 (7.2)
L2/SEA,0.3	Beijing/SIT1	13 (0.3)	10 (76.9)	3 (23.1)
L3/CAS, 21.7	L3. ETH1/SIT25	316 (7.2)	225 (71.2)	91 (28.8)
	Delhi/CAS	407 (9.3)	251 (61.7)	156 (38.3)
	L3.1.1/SIT21, SIT1675	150 (3.4)	132 (88)	18 (12)
	CAS	77 (1.8)	63 (81.8)	14 (18.2)
L4/EA, 62.3	H	447 (10.2)	277 (62)	170 (38)
	LAM	187 (4.3)	136 (72.7)	51 (27.3)
	T	573 (13.1)	409 (71.4)	164 (28.6)
	L4.6/SIT37	240 (5.5)	166 (69.2)	74 (30.8)
	L4.2. ETH1/SIT149	638 (14.6)	532 (83.4)	106 (16.6)
	L4.10/SIT53	423 (9.7)	320 (75.7)	103 (24.3)
	L4.1.1/X	73 (1.8)	59 (80.8)	14 (19.2)
	L4.4.1.1/S, SIT34	29 (0.7)	6 (20.7)	23 (79.3)
	Other L4	121 (2.8)	72 (59.5)	49 (40.5)
L7,3.4	L7	151 (3.4)	85 (56.3)	66 (43.7)
M.bovis, 0.5	*M. bovis*	22 (0.5)	10 (45.5)	12 (54.5)
Unclassified, 3.6	Unclassified	160 (3.7)	115 (71.9)	45 (28.1)
Total		4371 (100)	72.1	27.9

EAI: East African Indian, **SEA:** Southeast Asia, **CAS:** Central Asia; **EA:** Euro-American; **H:** Haarlem, **LAM:** Latin American and Mediterranean, **T:** Tuscany, **ETH:** Ethiopia, **S:** Sicily, **PTB:** Pulmonary tuberculosis, **TBLN:** Tuberculous lymphadenitis. **SIT:** Spoligotyping international type.

4. Discussion

The review summarized phylogenetic and phylodynamic characteristics of 4371 clinical isolates from thirty-one articles. Based on the pooled data investigated, the majority of cases were newly diagnosedPTB, male and within the age range of 15–44 years. The TB/HIV co-infection rate was 23.5%, similar to the recent review by Tesfaye et al. (2018) of 25.6% [3]. *M. tuberculosis* was responsible for 99.5% of TB in Ethiopia and M. bovis accounted for only 0.5% of cases.

Analysis of the national Mtb strain diversity shows the presence of all Mtb lineages except for L5 and L6 (*M. africanum*) in varying proportions in Ethiopia; from 62.3% for L4 to 0.3% for the L2. Fig. 2A and B displays the slight regional variations with regard to both (sub) lineages. This is in line with a systematic review of spoligotyping based articles in Ethiopia [51]. Phylogeography is shaped by the interplay between multiple interacting factors such as geography, demography, human migration [26], climate [91], genetic drift [92] and host-pathogen interaction [93]. However, which factors are the key drivers of Mtb strain regional variation in Ethiopia is not addressed and could be the subject of future research. Furthermore, geographic mosaic of human-Mtb coevolutionary model should also be deciphered in the future to better understand the phylogeographic nature of Mtb.

Out of 1939 shared-types (STs) identified by Brudy et al., 2006 [26], 259 (13.4%) SITs were identified in this nationwide review. Comas et al. (2015) concluded that the population structure of Mtb in Ethiopia is complex and diverse [29]. Moreover, homoplasy, recurrent mutation and recombination as a likely cause of diversity is excluded by another Comas et al. (2013) study [94]. Rather, consecutive entrance of multiple genotypes through human migration and trade was proposed as explanation for such high level of genetic diversity. Ethiopia has long history of contact and admixture with African and Eurasian population groups [95]. According to Comas et al., L3. ETH1 might have been introduced into Ethiopia through trade routes across the Indian Ocean. Moreover, a two-way north-south and south-north internal migration has been going on over centuries with a possible role in its further spread and diversity [29].

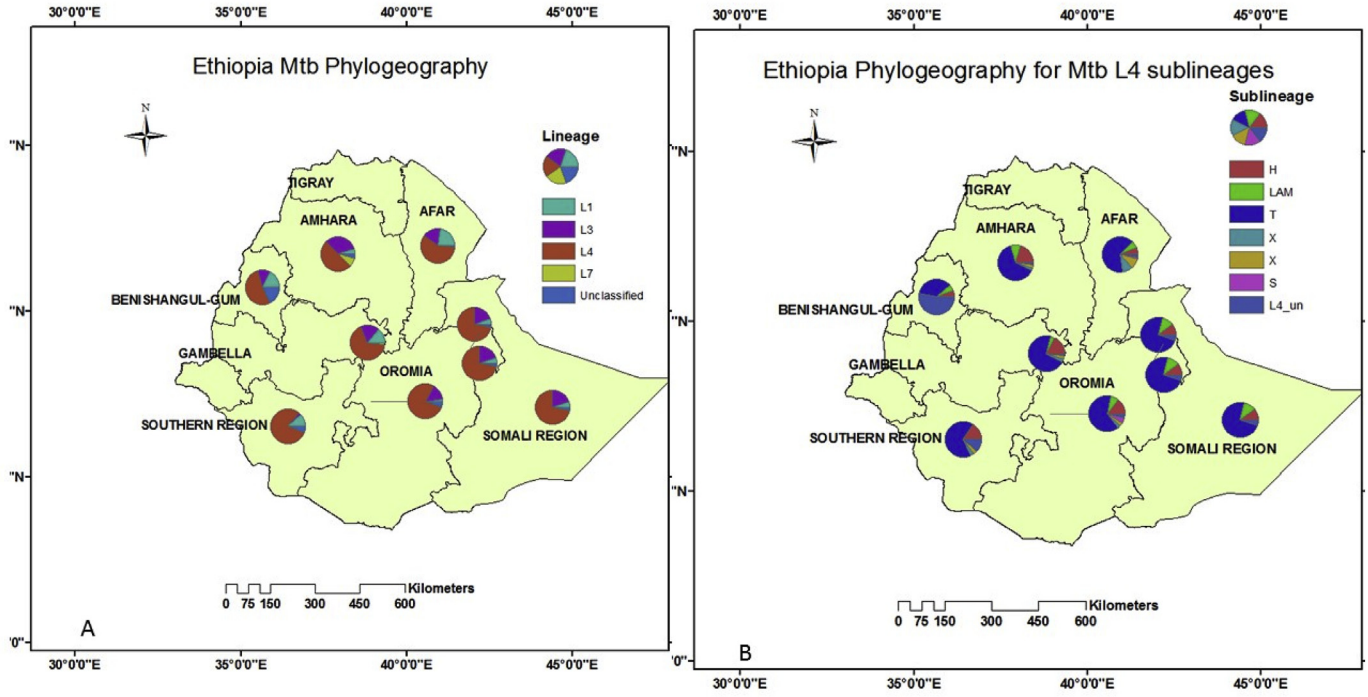

Fig. 2. *Geographic distribution of M. tuberculosis* lineage (A) and Euro-American sublineages (B) in Ethiopia, 2005–2018 (N = 4222).
EA: Euro-American, **LAM:** Latin American Mediterranean**, CAS:** Central Asia**; EAI:** East African Indian Lineage; ***: **Somali region data include** part of Haromaya, Dire-Dawa, Harari, Somali, Guji (Negele) and Liben (Filtu); Addis Ababa represent the central Ethiopia phylogeography.

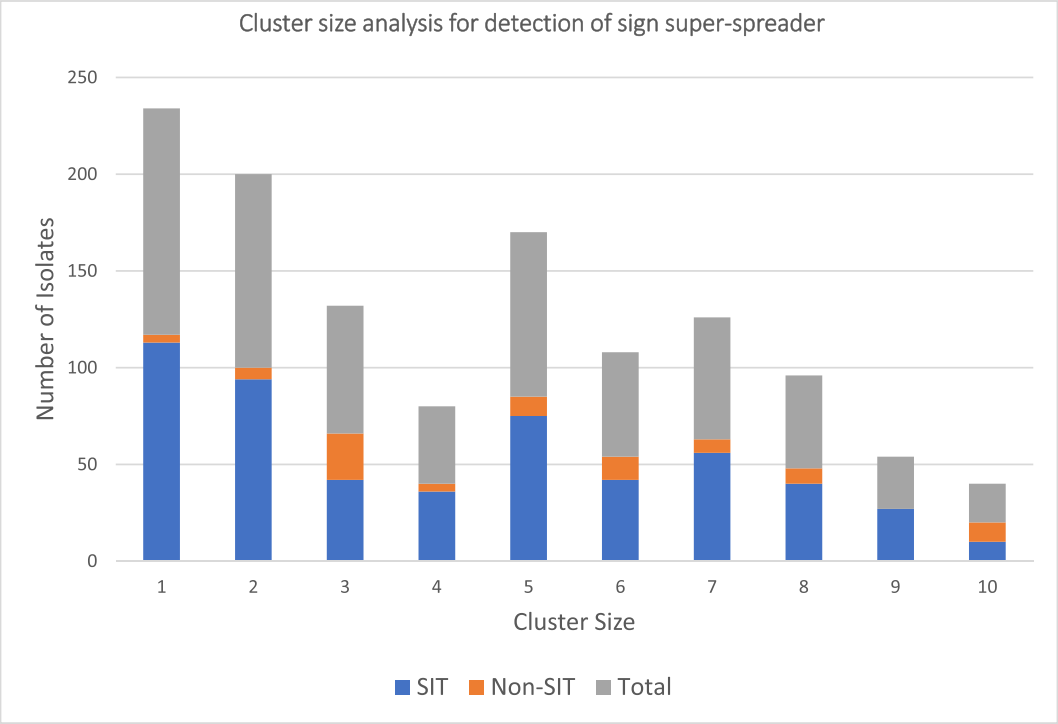

Fig. 3. The number of isolates with unique and clusters having ≤10 isolates, Ethiopia, 2005–2018.

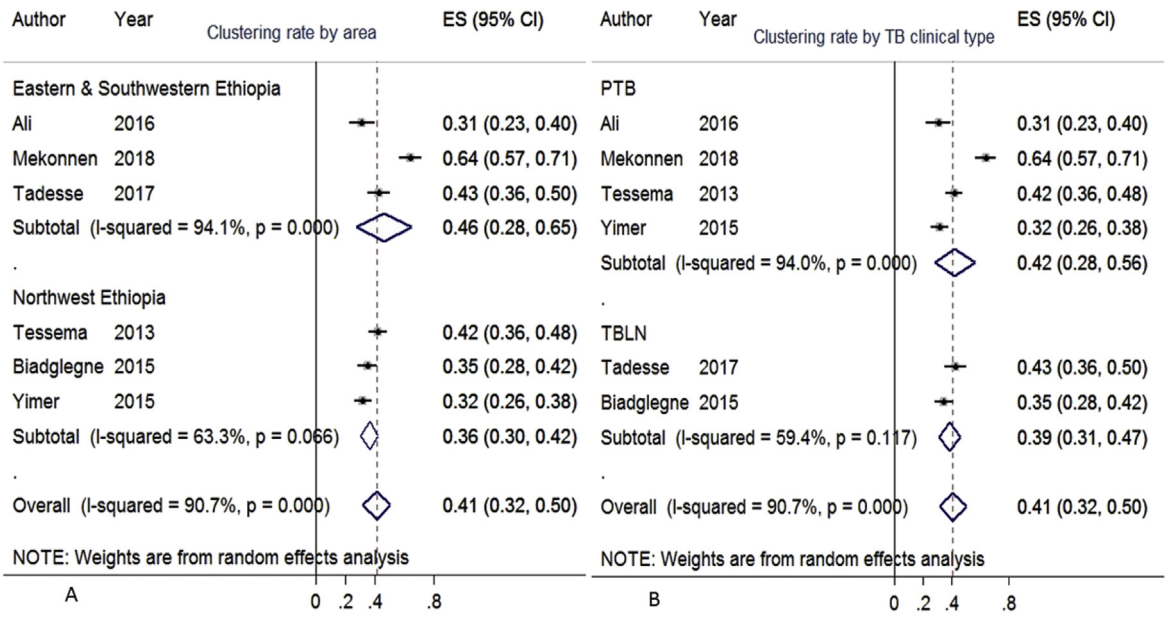

Fig. 4. Pooled clustering rate of *M. tuberculosis*, Ethiopia, 2005–2018.
The box from the forest plot indicated weight of articles from random effect analysis. The crossed line is the 95% CI, the solid vertical line is zero values to x-axis. Random effect cluster meta-analysis showed individual and pooled estimates stratified by area (A) and by TB clinical type (B).

Of the total 4371 isolates analyzed for transmission patterns, 3291 (75.3%) were grouped into 146 SIT clusters with cluster size ranges of 2–638 isolates per cluster (S2A). Specifically, L4.2. ETH1/SIT149, L4.10/SIT53, L3. ETH1/SIT25 and L4.6/SIT37 were isolates that had the largest cluster sizes (Table 3). Clustered strains were characterized by higher intracellular growth rate and hypo inflammatory phenotype which could facilitate higher transmission [27,96].These dominant SITs expected to be specialist strains to Ethiopia having sympatric association within the local community. The spread of TB is a branching process [8] and the presence of high heterogeneity and skewness in the number of secondary cases per infectious individual (Fig. 3) indicates the presence of super-spreaders [97]. Sympatric host-pathogen association is important for establishing longstanding carrier states and shedding of the pathogen for lengthy periods of time [98]. Super-shedding reflects the interaction of the host with the pathogen, whereas super-spreading reflects the interaction of the host with other hosts [99].

Based on MIRU-VNTR, the overall clustering and RTI was 41% and 29%, respectively. These two pooled prevalence rates were higher than the African average clustering rate of 30% and RTI of 20% [40].

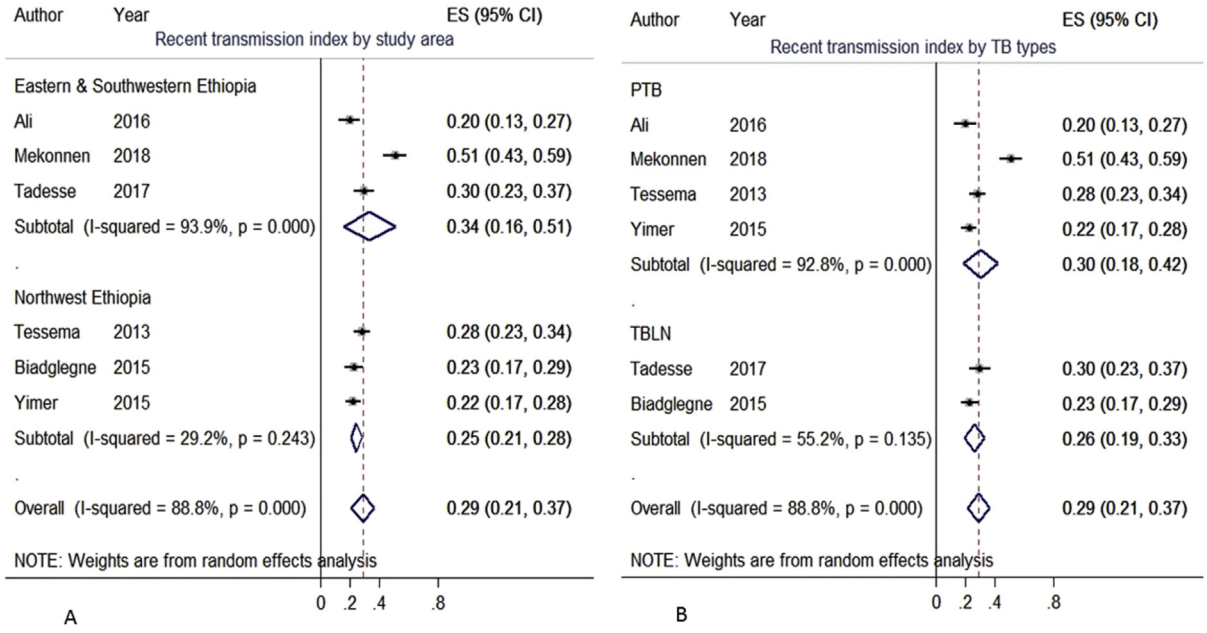

Fig. 5. Pooled recent transmission index of *M. tuberculosis*, Ethiopia, 2005–2018.
The box from the forest plot indicated weight of articles from random effect analysis. The crossed line is the 95% CI, the solid vertical line is zero values to x-axis. Random effect RTI meta-analysis showed individual and pooled estimates stratified by area (A) and TB clinical type (B).

Moreover, clustering and RTI were also higher in southwestern and eastern parts of Ethiopia than in the northwestern part of the country. This variation might be due to differences in the incidence of TB, host socio demographic status, study population and the strains [100]. Male gender and young adults were significantly more involved in clustering [101]. Variation in the strength of TB control and prevention packages such as the use of BCG (bacillus Calmette-Guerin) vaccine and isoniazid prophylaxis treatment (IPT), case finding and treatment strategy among regions might also affect rates of clustering [56]. Further, clustering is inversely related with HIV prevalence [102]. It would be interesting to investigate whether the HIV epidemic has impacted on clustering rates in Ethiopia, and whether the lower clustering rate in the northwest is thus related to the HIV epidemic, by comparing trends of HIV co-infection and clustering over time.

Of note is that, MIRU-VNTR or combined MIT and SIT could overestimate clustering and RTI compared to WGS based transmission analysis which uses five to twelve SNP difference as genetic relatedness [103].

Generalizability of these results is subject to certain limitations. First, the study did not include IS6110, large sequence polymorphism and SNP based articles. Second, articles were also retrieved from MEDLINE and Scopus only. Third, gray literature was not searched.

5. Concluding remarks

The Mtb population in Ethiopia is genetically diverse with lineages L4, L3, L1 and L7 making up 62.3%, 21.7%, 7.9% and 3.4% of strains, respectively. Among families, L4.2. ETH1/SIT149, L4.10/SIT53, L3. ETH1/SIT25 and L4.6/SIT37 were the leading clustered isolates. The dominance of a few genotypes argues in favor of the presence of super-spreader phenomena. L3/CAS and L7 shared 33% and 8% of the Amhara regional Mtb population structure. L1/EAI was proportionally higher in Afar. L4/EA was relatively higher in Oromia, Southern Nations and Nationalities Region, central Ethiopia and south eastern Ethiopia. The overall pooled clustering and RTI rate was 41% and 29%, respectively. The proportion of pooled clustering and RTI rates was higher in eastern and southwestern Ethiopia than in northwestern region. Ethiopia is one of the epicenters of TB in Africa. Spoligotyping and MIRU-VNTR are prone to convergent evolution and have low resolution power for cluster analysis. Thus, to better understand the evolution and transmission pattern of Mtb in Ethiopia and ultimately implement suitable TB control strategies tailored to the Ethiopian context, WGS based information would be needed.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets supporting the conclusions of this article are included within the article and its supplementary files.

Conflicts of interest

The authors declare that they have no competing interests.

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Authors' contributions

DM: Conceptualization, formal analysis, methodology, writing original draft, writing review & editing. AD: Data curation, methodology, writing original draft, writing review & editing. AC: Formal analysis, contribute software, participate in design. AS, FB, EN, AM: Participated in the data extraction, writing the draft and reviewing the final manuscript.AM, LW, KB, YK: Drafting the protocol, drafting and reviewing the final manuscript. SYA: Participated in the investigation of the draft, validation, drafting and reviewing of the final manuscript. TT:

Participated in supervision, validation and reviewing of the final manuscript. AA: Conceived the review topic, reviewed the protocol, supervised the review process, reviewed, investigated and validated the final manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tube.2019.101858.

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