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Hyper transmission of Beijing lineage *Mycobacterium tuberculosis*: Systematic review and meta-analysis

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SUMMARY

Objectives: The globally distributed "Beijing" lineage of *Mycobacterium tuberculosis* has been associated with outbreaks worldwide. Laboratory based studies have suggested that Beijing lineage may have increased fitness; however, it has not been established whether these differences are of epidemiological significance with regards to transmission. Therefore, we undertook a systematic review of epidemiological studies of tuberculosis clustering to compare the transmission dynamics of Beijing lineages versus the non-Beijing lineages.

Methods: We systematically searched Embase and MEDLINE before 31st December 2018, for studies which provided information on the transmission dynamics of the different *M. tuberculosis* lineages. We included articles that conducted population-based cross-sectional or longitudinal molecular epidemiological studies reporting information about extent of transmission of different lineages. The protocol for this systematic review was prospectively registered with PROSPERO (CDR42018088579).

Results: Of 2855 records identified by the search, 46 were included in the review, containing 42,700 patients from 27 countries. Beijing lineage was the most prevalent and highly clustered strain in 72.4% of the studies and had a higher likelihood of transmission than non-Beijing lineages (OR 1·81 [95% 1·28–2·57], $I^2 = 94·0\%$, $\tau^2 = 0·59$, $p < 0·01$).

Conclusions: Despite considerable heterogeneity across epidemiological contexts, Beijing lineage appears to be more transmissible than other lineages.

Introduction

Mycobacterium tuberculosis (Mtb) has a strictly clonal and hierarchical population structure, due to a near complete absence of horizontal gene transfer. The only apparent modes of evolution of modern strains are through single nucleotide substitution, deletion and duplication events.[1] Because of the clonal structure of Mtb, comparative genotypic analyses from diverse geographic populations can provide unique insights into dissemination dynamics and evolutionary genetics of the pathogen.[2]

Genotypic evaluation of strain relatedness is frequently used to complement epidemiological evidence of transmission. Genotyping can be performed using a variety of techniques that interrogate different classes of genetic markers and generate either strain-specific banding patterns (IS*6110* DNA fingerprint), bar code-like signals (spoligotyping), or numerical patterns (24 locus-MIRU-VNTR typing)[3] and most recently next generation whole genome sequencing (WGS) for genome-based epidemiology.[4] Increasing molecular identification in recent decades has raised questions regarding potential strain-specific differences in the clinical outcomes and epidemiological characteristics of Mtb infection.[1] Currently, seven lineages have been defined by unique event polymorphism (single nucleotide polymorphism or deletion). Most of these lineages are highly prevalent in specific geographic areas and are named according to their predominant geographical distribution: Lineage 1 (Indo-Oceanic lineage), Lineage 2 (East Asian; includes sub-lineage "Beijing"), Lineage 3 (CAS/ Delhi), Lineage 4 (Euro-American), Lineage 5 (West African 1) and Lineage 6 (West African 2), Lineage 7 (Ethiopia).[5,6] These phylogeographic distributions of Mtb lineages suggest local adaptation of the pathogen to sympatric human populations.

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Of particular interest has been a sub-lineage of Lineage 2 ("Beijing" strain), which is globally distributed[7] and has been associated with outbreaks.[8,9] In 2006, The European Concerted Action on New Generation Genetic Marker and Techniques for the Epidemiology and Control of Tuberculosis combined available datasets from all over the world (>29,000 patients from 49 studies in 35 countries) to assess the Beijing genotype's prevalence worldwide, trends over time and with age and its association with drug resistance.[10] Beijing lineage has been reported to be associated with an increased risk of acquired drug resistance, increased clinical severity and lesser protection from BCG vaccination.[7,11]

Laboratory based studies have also suggested that Beijing strains may have increased fitness,[12] although it has not been established whether these differences are of epidemiological significance with regards to transmission. Fitness of a transmissible organism can also be assessed by considering its effectiveness in terms of epidemic potential. Epidemic potential may be quantified by estimating the average number of secondary cases caused by a specific genotype after its introduction into an entirely susceptible population. These estimates rely on epidemiological evidence such as cluster studies, epidemiological investigation and model-based studies in human population rather than microbial behaviour in the laboratory because their precise contribution to the empiric success of an individual in the real world is not clear.[13] Therefore, we conducted a systematic review of epidemiological studies of Mtb transmission to quantify the extent of hyper-transmission of Beijing lineages.

Methods

Search strategy and selection criteria

We conducted a systematic review and meta-analysis of Mtb transmission to compare the epidemiological risk of transmission of Beijing versus non-Beijing lineage. Our search strategy was prospectively developed, recorded with the PROSPERO database (CDR42018088579) and conforms to the Preferred Reporting Items for Systematic reviews and Meta-analysis (PRISMA) guidelines.[14]

We searched two electronic databases for primary studies: MEDLINE and EMBASE until 31st of December 2018. Search terms included "tuberculosis", "*Mycobacterium tuberculosis*", "secondary cases", "secondary infection", "Beijing", "East-African Indian", "Euro-American", "West African 1", "West African 2", "Indo-Oceanic". The search was supplemented with additional search terms such as "fitness", "fitness cost", "strain"/"lineage" combined with terms for each lineage listed above, "transmission" and "transmission dynamics" to find relevant articles potentially missed during primary searching. We also incorporated a snowball sampling approach and hand searched articles identified from cross-references of identified articles and from suggestions of experts in the field. The study design involved observational studies (cross-sectional and longitudinal).

The titles and abstract for each of these citations were screened to capture relevant articles, with the following studies excluded: (1) studies not in English; (2) posters and reviews; (3) studies that lacked genotyping data; (4) studies related to *M. bovis* or *M. africanum* or non-tuberculous mycobacteria (5) studies focusing on immunological comparisons of plasma cytokine levels in peripheral blood mononuclear cells (6) proteomic approaches to understand the hypervirulence of Beijing isolates (7) studies which only involved multidrug resistant (MDR)-TB or extensively drug resistant (XDR)-TB patients (8) studies which focused on the single patient transmission chain and (9) studies limited to a single lineage only. Full text of the remaining citations was obtained and reviewed thoroughly against inclusion criteria. Disagreements between reviewers were resolved by consensus.

For an article to be included in the review, we required that the following information was reported: genotyping information for the patients with TB (pulmonary and/or extrapulmonary) relevant to the study irrespective of smear status, HIV status and age group. To account for recent transmission, a two-year cutoff period was considered ideal because it broadly coincides with the epidemiologically-observed high-risk period for the development of active TB after recent infection.[15–18] Using the 2-year cutoff period, an index case was defined as a pulmonary TB episode with a DNA fingerprint pattern that had not been assigned to another case within the preceding two years.[19] A secondary case was any case with an identical fingerprint pattern to the index case that was diagnosed no more than two years after the index case. We also investigated clustering information as it provides an indication of overall transmission leading to disease during the study period mentioned in each article. Included articles were required to provide information on either the number of secondary cases and index cases or the number of clustered cases, unique isolates and clusters for both Beijing and non-Beijing lineages. In all included studies, "cluster" was defined as ≥2 patients whose case isolates had identical DNA fingerprints. The percentage of recent transmission, which was our primary outcome measure, was calculated by the formula: $(n_c - c)/n$, where n is the total number of isolates, c is the number of clusters, and n_c is the total number of clustered isolates.[20–24] The clustering index was calculated by the total number of clustered isolates in each group divided by the total number of isolates for the group.[25]

Data analysis

The results of the electronic searches were compiled in Microsoft Excel and duplicate citations were removed. A data extraction form was used to extract the following information: authors, title, country of study, DOI, year of study, smear status of patients, number of secondary cases, number of index cases, transmission indices, number of index and secondary cases or number of clustered and non-clustered/unique isolates, HIV co-infection, resistance information for first-line drugs: isoniazid, rifampicin, ethambutol and pyrazinamide, age groups and conclusions.

Data were analysed using the meta-package[26] for the R programming language for statistical computing (version 3.2.3). We calculated pooled estimates of recent transmission, with their associated odds ratio (OR), standard error (95% CI), standard deviation (z) and p values for both fixed and random-effects models. Meta-analysis was done using the Mantel–Haenszel method; Hartung–Knapp adjustment for random effects model and Paule–Mandel estimator for τ^2. Continuity correction of 0.5 in studies with zero cell frequencies was used. Heterogeneity was assessed analytically by I^2 and Cochrane Q test.

Results

Systematic review

2843 articles were identified by the preliminary search strategy, with a further 12 articles identified from snowball sampling and manual review. After duplicate removal, 776 unique citations were identified, of which 504 publications were eligible for full text review and 46 met all eligibility criteria (Fig. 1).

The 46 included articles reported information on 42,700 patients diagnosed with tuberculosis from 27 countries. Various molecular genotyping methods were used, providing information on clustering by genotype. Table 1 presents an overview of the different molecular typing techniques used. We included twelve studies that used IS*6110*-RFLP for typing, twenty-eight studies that used spoligotyping, thirty-two studies that used MIRU-VNTR

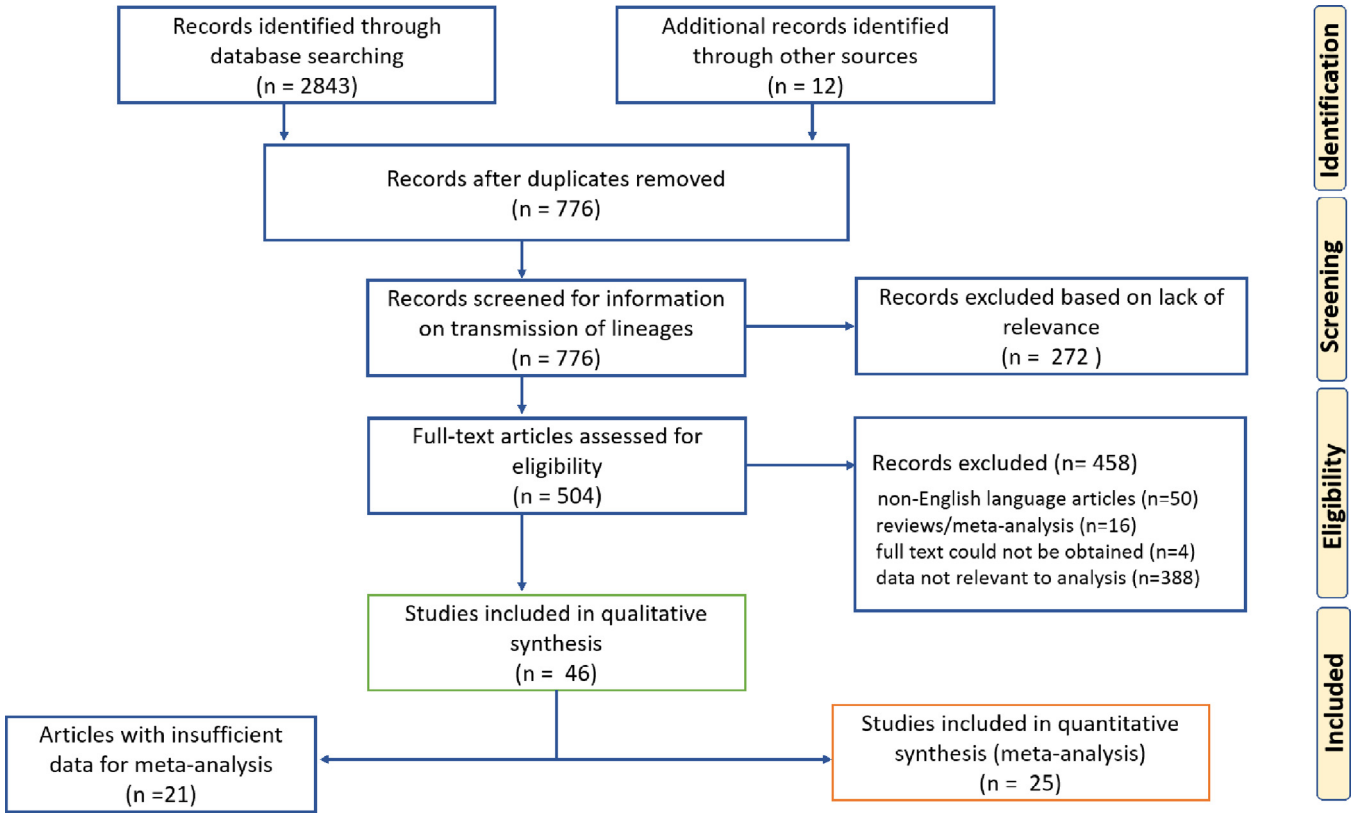

Fig. 1. Flow diagram of the study selection.

Table 1
Characteristics of included studies.

Study	Country	Region	Sample size	Year	Molecular typing	Findings
Anh et al.[27]	Vietnam	Ho Chi Minh City	563	2000	Spoligotyping	Beijing lineage constituted 53.5% of total isolates
Caminero et al.[28]	Spain	Gran Canaria Island	651	2001	IS*6110*-RFLP Spoligotyping	Beijing lineage constituted of the largest cluster (75 cases)
Banu et al.[29]	Bangladesh	Dhaka City	48	2004	Spoligotyping MIRU-VNTR	Beijing lineage constituted 31.3% of total isolates, of which 73.3% were clustered
Cox et al.[30]	Uzbekistan and Turkmenistan	Karakalpakstan, Dashoguz Velayat	382	2005	IS*6110*-RFLP Spoligotyping	Beijing constituted of 50.0% of the total isolates, of which 55.0% were clustered
Drobneiwski et al.[31]	Russia	Samara Region	880	2005	Spoligotyping 12 MIRU-VNTR	Beijing constituted of 63.4% of the total isolates
Hasan et al.[32]	Pakistan	Karachi, Punjab Province, Sindh Province, Northwest Frontier Province and Balouchistan Province	314	2006	Spoligotyping	Beijing constituted of 6.0% of total isolates of which 9.0% were clustered; Lineage 3 constituted of 39.0 of isolates
Dou et al.[33]	Taiwan		208	2008	Spoligotyping, 19 MIRU-VNTR, NTF loci typing and RD deletion number determination	Beijing lineage was the most prevalent, and was present in 40.0% of specimens from the aboriginal population, 72.4% of veterans, and 56.0 of the general population
Cowley et al.[34]	South Africa	Cape Town	291	2008	Spoligotyping	Beijing constituted 23.4% of total isolates
Mokrousov et al.[35]	Russia	Kaliningrad	90	2008	12 MIRU-VNTR	Beijing constituted of 41 of 90 isolates, representing the largest cluster (45.6%)
Van der Spuy et al.[36]	South Africa	Cape Town, Western Cape	1920	2009	IS*6110*-RFLP	Beijing constituted 39.2% of the total isolates of which 81.8% were clustered cases

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Table 1 (*continued*)

Study	Country	Region	Sample size	Year	Molecular typing	Findings
Pardini et al.[37]	Georgia	Abkhazia	311	2009	IS*6110*-RFLP Spoligotyping	Beijing constituted 25.1% of total isolates and was significantly associated with clustering (OR 2.7)
Parwati et al.[38]	Indonesia	Jarkata and Bandung	844	2010	Spoligotyping	Beijing constituted 33.4% of isolates
Hu et al.[39]	China	Deqing County in Zhejiang Province and Guanyun County in Jiangsu Province (eastern China)	399	2010	IS*6110*-RFLP	Beijing constituted 80.1% of all isoniazid-resistant isolates, of which 56.2% were clustered
Shamputa et al.[40]	South Korea		208	2010	24 MIRU-VNTR Spoligotyping	Beijing constituted 97.1% of total isolates, but the clustering rate was low (22.3%)
Gallego et al.[41]	Australia	New South Wales	855	2010	12 MIRU-VNTR Spoligotyping	Beijing constituted 24.0% of total isolates along with the cluster having the highest number of isolates (49)
Wang et al.[42]	China	Heilongjiang Province	200	2011	Spoligotyping, Beijing family specific PCR, 19 MIRU-VNTR	Beijing lineage represented 89.5% of all isolates, of which 16.8% were clustered
Weisenberg et al.[25]	USA	New York City	3911	2012	IS*6110*-RFLP	Beijing constituted 15.1% of total isolates, of which 23.9% were clustered
Buu et al.[43]	Vietnam	Tien Giang Province (Southern Vietnam)	2207	2012	IS6110-RFLP	Beijing constituted 35.6% of total isolates, of which 37.0% were clustered; Lineage 1 constituted of 67.0 of clustered isolates
Aleksic et al.[44]	Kiribati	South Tarawa	74	2013	24 MIRU-VNTR IS*6110*-RFLP Spoligotyping	Beijing constituted 49.0% of total isolates of which 62.8% were clustered
Al-Hajoj et al.[45]	Saudi Arabia		902	2013	Spoligotyping 24 MIRU-VNTR	Beijing constituted 5.8% of all isolates, of which 55.8% were clustered
Langlois-Klassen et al.[46]	Canada	Alberta	1397	2013	IS*6110*-RFLP Spoligotyping	Beijing constituted 19.0% of all isolates, of which 21.0% were clustered
Lu et al.[47]	China	Jiangsu Province	497	2014	Spoligotyping 15 MIRU-VNTR	Beijing constituted 81.1% of all isolates, of which 32.5% were clustered
Liu et al.[48]	China	Gansu Province	426	2014	Spoligotyping 15 MIRU-VNTR	Beijing constituted 87.6% of all isolates and the largest cluster
Liu et al.[20]	China	Jiangsu Province	441	2014	Seven loci MIRU-VNTR (3820, Qub11a, Qub11b, Qub18, Qub26, MIRU26 and Mtub21)	Beijing constituted 89.3% of all isolates, but the clustering rate was low (4.4%)
Chen et al.[47]	Taiwan		177	2014	Spoligotyping and 24 MIRU-VNTR	Beijing constituted 35.2% of all isolates, of which 42.9% were clustered
Gurjav et al.[23]	Australia	Sydney, New South Wales	1128	2014	24 MIRU-VNTR	Beijing constituted 27.6% of all isolates, of which 40.5% were clustered
Zmak et al.[49]	Croatia		1587	2014	15 MIRU-VNTR	Lineage 4 constituted 66.7% and Beijing constituted 0.1% of the total isolates
Yang et al.[50]	China	Five sites	2274	2015	Different sets of MIRU-VNTR, hypervariable VNTR loci (3820, 1982, 3232 and 4120)	Beijing strain were more likely to be clustered (OR 1.67)
Yuan et al.[51]	China	Xinjiang Province	381	2015	24 MIRU-VNTR	Beijing constituted 57.5% of all isolates, of which 11.9% were clustered
Mathema et al.[52]	South Africa	15 mines (Gauteng, North West, and Free State)	1240	2015	IS*6110*-RFLP	Beijing constituted 13.6% of all isolates and most of the large clusters
Barletta et al.[53]	Peru	Lima	844	2015	Spoligotyping 15 MIRU-VNTR	Beijing constituted 16.4% of total isolates of which 59.2% were clustered (Lineage 4 was predominant)
Nebenzahl-Guimaraes et al.[54]	Netherlands		4436	2015	Spoligotyping 24 MIRU-VNTR	Beijing constituted 12.8% of total isolates of which 29.7% were clustered (Lineage 4 was predominant)

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Table 1 (*continued*)

Study	Country	Region	Sample size	Year	Molecular typing	Findings
Globan et al.[55]	Australia	Victoria	2377	2015	15 MIRU-VNTR 24 MIRU-VNTR	Beijing constituted 20.7% of total isolates of which 80.9% were clustered
Hu et al.[56]	China	six rural counties	1222	2016	24 MIRU-VNTR Spoligotyping	Beijing constituted 79.1% of all isolates, of which 22.6% was clustered
Gurjav et al[24]	Australia	New South Wales	1692	2016	24 MIRU-VNTR WGS	Beijing constituted 27.8% of total isolates of which 35.7% were clustered
Liu et al.[57]	China	Beijing	679	2017	Spoligotyping 12 MIRU-VNTR	Beijing constituted 81.7% of total isolates of which 45.2% were clustered
Murase et al.[58]	Japan	37 prefectures	981	2017	28 MIRU-VNTR	Beijing constituted 70.6% of isolates of which 77.0% were clustered
Lalor et al.[59]	England		1646	2017	24 MIRU-VNTR	No increased clustering in the Beijing lineage compared to non-Beijing (increased transmission in Lineage 4 and CAS observed)
Liu et al.[60]	China	Xinjiang	311	2017	15 MIRU-VNTR Spoligotyping	Beijing constituted 72.0% of all isolates, of which 60.3% were clustered
Sharma et al.[61]	India	Ghatampur, Agra	355	2017	Spoligotyping 12 MIRU-VNTR	Beijing constituted 3.9% of all isolates, of which 3.0% were clustered; Lineage 3 was predominant
Riyahi Zaniani et al.[62]	Iran	Isfahan	49	2017	15 MIRU-VNTR	Beijing constituted 24.4% of all isolates, while Lineage 4 constituted 44.9% of isolates; overall low clustering rates
Yamamoto et al.[63]	Japan	Airin area, Osaka City	596	2018	24 MIRU-VNTR	Beijing constituted 80.3% of all isolates, of which 41.8% were clustered
Liu et al.[64]	China	Beijing	1189	2018	Spoligotyping VNTR typing	Beijing constituted 83.3% of isolates and was significantly associated with clustering (22.7%)
Holt et al.[65]	Vietnam	Districts 1, 4, 5, 6 and 8, Tan Binh, Binh Thanh and Phu Nhuan	1635	2018	WGS	Beijing constituted 59.0% of isolates of which 31.5% were clustered
Uddin et al.[66]	Bangladesh	Mymensingh, Netrokona, Kishoreganj, Jamalpur and Tangail districts (northeast part of Bangladesh)	244	2018	Spoligotyping 12 MIRU-VNTR	Beijing constituted of 7.4% of all isolates and Lineage 1 constituted of 27.0%
Bainomugisa et al. [67]	Papua New Guinea		100	2018	WGS	95 out of 100 clinical isolates typed belonged to Beijing stain

Notes: IS*6110*-RFLP – Restriction Fragment length polymorphism targeting the insertion sequence IS*6110*.
MIRU-VNTR – Mycobacterial Interspersed Repetitive Units (MIRU) specific multiple locus Variable Number of Tandem Repeats (VNTR) analysis.
WGS – Whole Genome Sequencing.
PCR – Polymerase Chain Reaction.
NTF – 556 bp of intervening sequence.
RD – Regions of differences.

typing and three studies that used WGS. Twenty-five studies used multiple methods of molecular typing to investigate the genotypic diversity of Mtb isolates. Studies included in the review were from a wide range of geographical settings, including nineteen high and eight low incidence settings.

Beijing lineage constituted the greatest proportion of total isolates in thirty-three of the forty-six studies (71.7%) included in the review (Table 1). Nineteen out of twenty-six studies (73.1%) had a higher clustering index for Beijing than non-Beijing strains (Table 2). Eleven studies had recent transmission rates that were higher for Beijing and three out of four studies which reported the mean number of secondary cases (transmission index) observed higher numbers in Beijing clusters; therefore 77.8% of the studies had a higher primary outcome measure for Beijing (Table 3).

Longitudinal reporting from several countries has found that Beijing strains constituted a growing proportion of total cases [43,53,68]. High rates of ongoing transmission of Beijing were seen in high-incidence settings, including Kiribati,[44] Saudi Arabia,[45] Vietnam,[27,43,65] India,[61,69] Spain,[28] Bangladesh,[29] Taiwan,[33,70] Uzbekistan and Turkmenistan,[30] Russia,[31,35] China,[39,47,50,56,57,64] Japan,[58,63] Georgia,[37] Estonia,[68] Indonesia,[38] South Africa [36] and one low-incidence setting, the Netherlands.[54] Low level transmission was observed in Australia, with clustering analysis revealing that the largest clusters comprised of Beijing lineage.[24,41,55] However, Beijing lineage did not show increased transmissibility compared to non-Beijing lineage in other settings with comprehensive and effective TB prevention and care practices, including the United Kingdom [59] and Canada.[46] In Pakistan it was observed that Beijing was well established in the region and was not a result of recent transmission.[32] Low levels of transmission were also observed in South Korea [40] and in certain rural areas of China.[20,42,51] In South African pediatric [71] and goldmining [52] populations no significant association was found between Beijing lineage and recent transmission.

Beijing and its association with age

Clustering of Beijing lineage in younger age groups is particularly likely to reflect recent transmission. 60.0% of the studies

Table 2
Clustering percentages reported or calculated in different studies for Beijing and non-Beijing strains (* reported in the study).

Study	Year of study	Beijing proportion clustered (%)	Non-Beijing proportion clustered (%)
Anh et al.[27]	2000	53.46	46.53
Cox et al.[30]*	2005	54.73	25.00
Duo et al.[33]	2008	75.63	41.37
Van der Spuy et al.[36]	2009	81.81	59.89
Hu et al.[39]	2010	56.19	15.38
Wang et al.[42]*	2011	16.80	0.00
Buu et al.[43]	2012	37.15	45.32
Weisenberg et al.[25]*	2013	34.80	31.30
Langlois-Klassen et al.[46]	2013	21.31	37.28
Al-Hajoj et al.[45]*	2013	55.76	34.65
Aleksic et al.[44]*	2013	62.79	37.20
Liu et al.[20]	2014	8.07	7.27
Chen et al.[47]	2014	57.14	32.09
Barletta et al.[53]*	2015	59.23	71.71
Nebenzahl-Guimaraes et al.[54]*	2015	32.00	27.50
Globan et al.[55]	2015	17.20	27.63
Yuan et al.[51]*	2015	11.87	24.69
Yang et al.[50]	2015	80.85	71.63
Hu et al.[56]*	2016	22.60	7.80
Liu et al.[57]*	2017	45.21	28.57
Murase et al.[58]	2017	22.68	77.31
Liu et al.[60]*	2017	60.27	25.29
Sharma et al.[61]	2017	2.99	10.96
Yamamato et al.[63]*	2018	41.33	36.75
Liu et al.[64]*	2018	22.70	9.00
Holt et al.[65]*	2018	31.50	14.00

Table 3
Recent transmission proportions reported or calculated in different studies for Beijing and non-Beijing strains (* reported in the study).

Recent transmission			
Study	Year of study	Beijing (%)	Non-Beijing (%)
Duo et al.[33]	2008	52.81	36.60
Van der Spuy et al.[36]*	2009	73.00	45.20
Wang et al.[42]*	2011	10.00	0.00
Weisenberg et al.[25]*	2013	23.90	25.68
Gurjav et al.[23]*	2014	26.90	6.20
Liu et al.[20]*	2014	4.43	3.99
Chen et al.[47]	2014	88.88	32.11
Barletta et al.[53]	2015	53.80	57.33
Yuan et al.[51]	2015	5.47	11.11
Gurjav et al.[24]*	2016	24.30	8.60
Liu et al.[57]*	2017	45.21	28.57
Liu et al. [60]*	2017	45.53	16.09
Liu et al.[64]	2018	20.52	5.52
Yamamato et al.[63]	2018	18.42	30.72
Transmission index (mean number of secondary cases)			
Study	Year of study	Beijing	Non-Beijing
Langlois-Klassen et al.[46]*	2013	0.06	0.14
Globan et al.[55]	2015	7.29	2.96
Nebenzahl-Guimaraes et al.[54]*	2015	1.18	1.02
Lalor et al.[59]*	2017	2.17	1.76

included in the review observed a strong association between clustering and younger age among the Beijing strains. For example, a greater degree of clustering was observed in the 25–44 year age group for studies undertaken in China,[20,50,64] Japan[58] and Indonesia[38]; the cross-sectional study from South Africa done in 15 gold mines across three provinces showed highest clustering among the 45–54 year age group[52]; and in Estonia the majority of clustered cases occurred in individuals aged 30–39 years.[68] A study from Saudi Arabia[45] found that Beijing was distributed equally among all age groups. In Australia[55] and other low incidence countries like Netherlands,[54] Beijing was the most common genotype among young adults (15–29 years old) and in the elderly (<60 years old).[23,24]

Beijing and its association with drug resistance

While the focus of this review is on transmissibility, the presence of drug resistance in isolates may be relevant to risk of secondary infection. We therefore summarise data on drug resistance in the studies identified by our systematic review. Thirty-three out of forty-six included studies reported associations between specific lineages and drug resistance, of which twenty studies (60.6%) showed significantly higher proportion of drug resistance among Beijing lineage. In studies conducted in China, some found higher rates of drug resistance among Beijing strains,[39,50,72] while others found no difference.[42,47] In the Taiwanese aboriginal population, a strong association was found between Beijing and MDR-TB.[47] 39.0% of the Beijing isolates (97.1% of the total isolates) found in South Korea were from MDR-TB or XDR-TB patients.[40] The association between MDR-TB and Beijing genotype in Vietnam was strongly associated with resistance to streptomycin.[43,65] Considerable ongoing transmission of MDR-TB strains of the Beijing lineage was observed in India,[61,69] Bangladesh,[66] Pakistan,[32] Papua New Guinea,[67] Russia,[31,35] Georgia,[37] Uzbekistan and Turkmenistan.[30] In Australia, the number of cases of MDR-TB was small and rates of drug resistance were unchanged since the 2006; however, the Beijing strain was found to be associated with a higher incidence of drug resistance.[23] We also found studies which reported no significant difference in drug resistance distribution between Beijing and non-Beijing lineages.[42,43,47,51,53,56,57,64] In the South African gold miner population, the AH strain (X family) was found to be associated with drug resistance and outbreaks.[52]

Meta-analysis

Following assessment of clustering and transmission indices of the different studies included in the review, we proceeded to our pre-planned meta-analysis. Twenty-five articles had information to conduct the meta-analysis. The odds ratio for the fixed effects model was 1.48 (95% CI 1.38 to 1.58, $z = 11.78$ $p < 0.0001$), while the odds ratio for the random effects model was 1.81 (95% CI 1.28 to 2.57, $z = 3.53$, p 0.0017) (Fig. 2). There was an even contribution

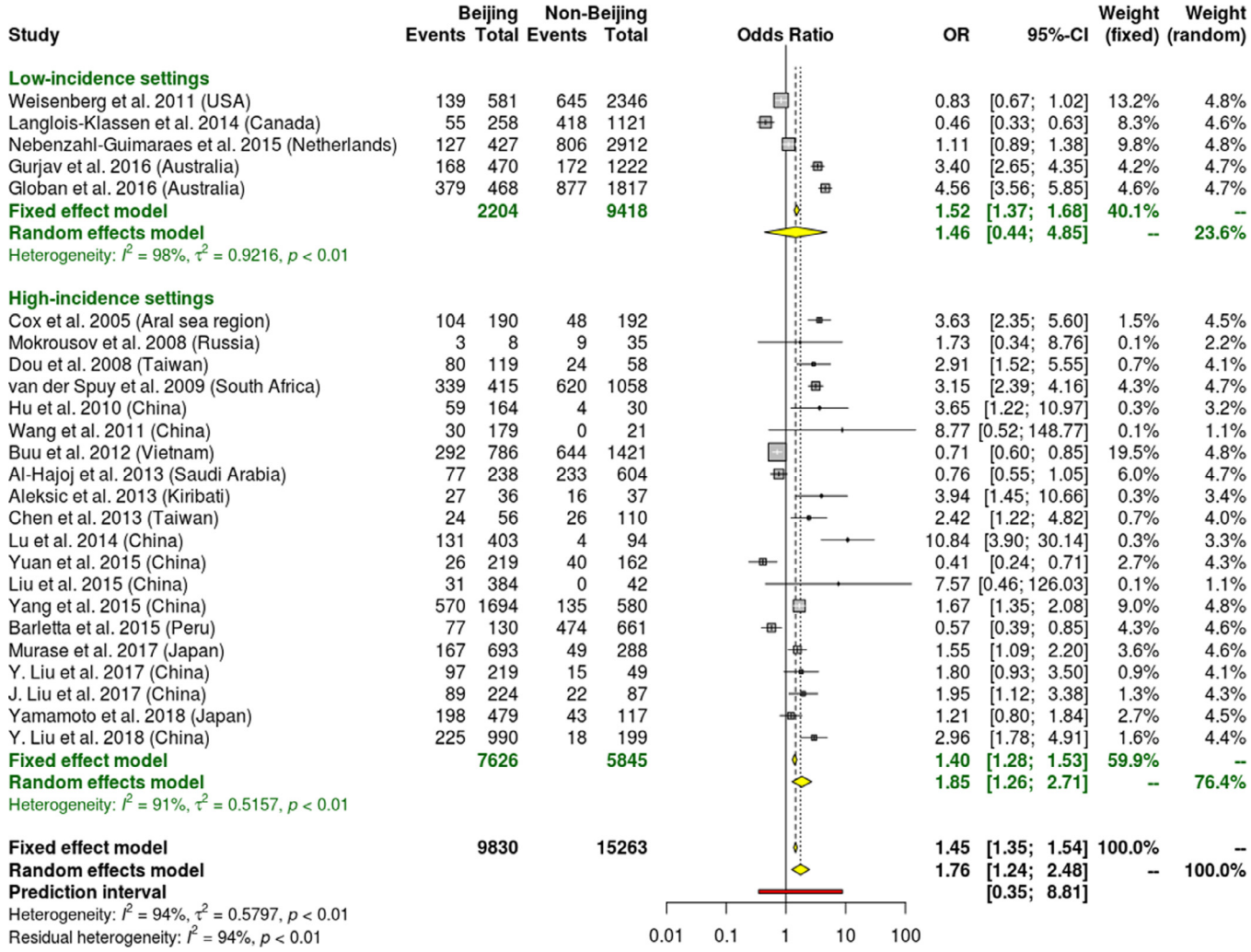

Fig. 2. Forest plot displaying the pooled estimates of transmission for Beijing and non-Beijing strains: The studies have been arranged in chronological order according to their date of publication. The first five studies are from low-incidence setting and the remaining 20 studies are from high-incidence setting.

from each included study (approximately 4% weight for each), but statistical heterogeneity was high.

Discussion

We found that Beijing lineage of Mtb was more likely to be associated with transmission than non-Beijing lineages. The strength of the observed relationship between Beijing lineage and transmission (OR 1.81) was notable and reflects a finding likely to be of epidemiological significance.

While our report has identified a statistically significant association between Beijing lineage TB and transmission, the mechanism for such an effect is inadequately understood. This finding may reflect either the selection of defined sub-lineages in different geographical settings, or the adaptation of strains in a defined Mtb sub-lineage capable of spreading more readily in certain human populations. It seems plausible that evolutionarily modern lineages like Beijing induce weaker immune response than ancient lineages, and this response potentially provides modern lineages with a selective advantage in terms of more rapid disease progression and/or transmission in human populations.[73] However, influence on transmission from microbiological fitness, differential immune response, or other mechanisms also remain plausible explanations. As observed in several studies East-African Indian (EAI) lineage was associated with notably low clustering rates, suggesting they are less likely to be transmitted, raising the possibility of future strain replacement.[54,74] Also, the frequency of transfer between diverse population groups like Vietnam[65] and Eastern Europe[37] supports previous assumptions that the Beijing lineage is a host generalist, capable of moving between ethnically diverse host populations.[9,48]

Our study's strengths include its systematic nature and emphasis on epidemiologic transmissibility, and our findings are limited by the heterogeneity of outcomes and variation in epidemiological and genomic definitions adopted. Classical molecular genotyping has been nearly used for thirty years to define transmission chains / clusters, but it comes with an inherent limitation: overestimation of recent transmission events.[24,42] Spoligotyping has lower discriminatory power compared to MIRU-VNTR; however, a combination of both shows better discriminatory power.[75] Majority of the studies included in the review used both Spoligotyping and MIRU-VNTR as genotyping methods to determine clusters. Studies that only used Spoligotyping were not included in the meta-analysis to avoid overestimation of recent transmission. If these studies were further paired with whole genome sequencing-based approaches the extent of overestimation could be refined further.[76] With the ever-decreasing cost of whole genome sequencing and easier implementation in a variety of settings (especially high-incidence, low-resource settings), it is likely to become

an integral part of the epidemiological approach to track and stop TB.

The high genotypic diversity seen in a low-incidence setting like Australia reflect the large number of overseas-born patients who migrate from all over the world rather than local transmission.[24,41,55] By contrast, studies from Asia and Russia highlighted the high levels of genome homoplasy within the Beijing strain family.[23,40,58] In high endemic settings like India,[61] Taiwan[33] and South Africa,[52] the high genetic diversity of the bacillary load could be explained by a mobile population in combination with reactivation, appearance and disappearance of individual clones and the long incubation period of the disease. Socio-demographic factors like lack of permanent housing, which leads to congregation of people in specific locations and spreading the infection, was observed as a correlate of clustering in Estonia.[68] We also think that it is unlikely a founder effect has a significant role in apparent clustering of Beijing lineage for several reasons. First, historically substantial shifts have been seen in lineage distribution in recent decades, suggesting a dynamic environment where transmission between regions remains relevant. Second, we have included studies where Beijing is both a majority and minority strain, minimising the potential impact of a founder effect. Finally, we have also included a two-year cut off period for defining clustering, which should also be helpful in concentrating the effect seen towards recent transmission.

The definition of fitness includes a microorganism's ability to survive, reproduce and to be transmitted.[13] Mutations leading to drug resistance development may influence the fitness of the microorganism. It has been speculated that low physiological cost of rifampicin resistance and compensatory mutations restoring fitness of Mtb maybe responsible for the widespread dissemination of the Beijing strain.[12] It was also observed that Beijing strains that were MDR were universally resistant to streptomycin.[43,58,65] An association between Beijing lineage and the development of drug resistance could influence clustering of isolates. This is expected to result in a selective advantage for Beijing strain and therefore would lead to higher prevalence of Beijing.[30] The hypervirulence of Beijing strains can be attributed to deletions in *ppe38*, which is responsible for the secretion of a subset of ESX-5 substrates.[77]

This review reinforces the epidemiological significance of Mtb lineages and highlights the importance of combining optimal molecular strain typing with epidemiological data. Further research into the mechanisms of increased transmissibility is required and translating genotypic data into programmatic algorithms. Mathematical models of TB transmission incorporate the process of infection; interventions leading to faster diagnosis and therefore reduced transmission.[78] Effective reproductive number (R_e), which represents the average number of secondary cases arising from a primary case of active TB is commonly used to describe infectiousness. In our current review, we are unable to estimate fully the *Re* from the available data, because our analysis only considers clustered events separated by less than two years and its well-known that late reactivation episodes after this time period are important in sustaining transmission of Mtb. Although a value of one is an important *Re* threshold for disease persistence in a population in general, the relative magnitude of *Re* for two co-circulating strains is of greater relevance to which Mtb strain will be sustained within a population. For outbreaks of a single pathogen, heterogeneous transmission has been shown to favour stochastic extinction as well as explosive outbreaks.[79] Given the high heterogeneity of TB transmission,[80,81] similar principles may apply to a multi-strain competition, in which one strain may replace another more rapidly than predicted by models that assume well-mixed populations. This may explain some of the heterogeneity in our findings.

Expansion of genotyping techniques holds great promise for optimizing public health management of TB. Inclusion of clustering information in routine public health responses is already used for tailoring strategies to reduce Mtb transmission and reactivation. Our results suggest that strategies enhancing contact tracing towards Beijing lineages could be evaluated further, particularly in high incidence settings where they are likely to contribute most to onward transmission and perpetuating the global TB epidemic.

Declaration of Competing Interest

None.

CRediT authorship contribution statement

Malancha Karmakar: Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing - original draft. **James M. Trauer:** Methodology, Investigation, Writing - review & editing. **David B. Ascher:** Supervision, Writing - review & editing. **Justin T. Denholm:** Conceptualization, Data curation, Investigation, Funding acquisition, Project administration, Supervision, Writing - review & editing.

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Supplementary materials

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