

BMJ Open Effect of study design and setting on tuberculosis clustering estimates using Mycobacterial Interspersed Repetitive Units-Variable Number Tandem Repeats (MIRU-VNTR): a systematic review

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ABSTRACT

Objectives: To systematically review the evidence for the impact of study design and setting on the interpretation of tuberculosis (TB) transmission using clustering derived from Mycobacterial Interspersed Repetitive Units-Variable Number Tandem Repeats (MIRU-VNTR) strain typing.

Data sources: MEDLINE, EMBASE, CINAHL, Web of Science and Scopus were searched for articles published before 21st October 2014.

Review methods: Studies in humans that reported the proportion of clustering of TB isolates by MIRU-VNTR were included in the analysis. Univariable meta-regression analyses were conducted to assess the influence of study design and setting on the proportion of clustering.

Results: The search identified 27 eligible articles reporting clustering between 0% and 63%. The number of MIRU-VNTR loci typed, requiring consent to type patient isolates (as a proxy for sampling fraction), the TB incidence and the maximum cluster size explained 14%, 14%, 27% and 48% of between-study variation, respectively, and had a significant association with the proportion of clustering.

Conclusions: Although MIRU-VNTR typing is being adopted worldwide there is a paucity of data on how study design and setting may influence estimates of clustering. We have highlighted study design variables for consideration in the design and interpretation of future studies.

INTRODUCTION

The introduction of molecular typing methods has improved our understanding of *Mycobacterium tuberculosis* (TB) transmission and has changed local and national control policies.^{1–5} The proportion of cases that are clustered is often used to estimate the amount of ongoing transmission within the population, based on the assumption that

Strengths and limitations of this study

- This is a timely evaluation of the impact of study design on estimates of tuberculosis clustering using Mycobacterial Interspersed Repetitive Units-Variable Number Tandem Repeats strain typing because it has been incorporated into national typing services globally.
- The strength of this meta-analysis was limited by the lack of detail reported by the included studies, highlighting the need for better quality reporting in primary studies.
- We have shown that the proportion of clustering derived from MIRU-VNTR typing is influenced by the number of loci typed, whether consent is required to type isolates, TB incidence in the study setting, and the maximum cluster size, highlighting these as important considerations in the design and interpretation of future studies.

cases with indistinguishable strain types are part of a chain of transmission. TB molecular typing methodology is changing rapidly and it is important that we better understand how to interpret the outputs and thus act.

TB molecular typing methods include Spoligotyping,⁶ insertion sequence 6110 (IS6110) restriction fragment length polymorphism (RFLP) analysis (the recent gold standard),⁷ Mycobacterial Interspersed Repetitive Units-Variable Number Tandem Repeats (MIRU-VNTR) typing,⁸ and whole genome sequencing.^{9–11} Published reviews have identified factors that might influence or bias clustering by IS6110 RFLP.^{12 13} No study has repeated this analysis using more up-to-date typing methods, which is important for understanding of the epidemiology of TB and to shape the application of molecular typing to improve TB control.

Published meta-analyses and modelling studies using *IS6110* RFLP data show that the proportion of clustering observed can be affected by (1) study design (affecting the proportion of eligible cases that are included in the study); (2) features of the typing method (such as the ability to type isolates with low copy numbers); and (3) study setting (such as characteristics of the study population). For example, the proportion of clustering increases when the fraction of the total data sampled increases^{13–15} and when study duration increases.¹⁶

MIRU-VNTR is currently the preferred method of molecular typing,^{17–21} and can be used together with Spoligotyping.⁸ Relative to *IS6110* RFLP, MIRU-VNTR does not have to exclude isolates with a low *IS6110* copy number, has a faster turnaround time, is high throughput and the numeric strain types are more easily compared. MIRU-VNTR strain typing is increasingly being adopted worldwide,^{1 22–27} yet unlike *IS6110* RFLP, the evidence for the interpretation of the findings such as the impact of study design and setting on clustering have not been reviewed. Although the two typing methods have been shown to have a similar discriminatory value, the markers evolve independently and at different rates, resulting in a difference in clustering between the two methods.²⁸ This suggests that there could be differences in the way study design, typing method and setting affects clustering by the two methods. We conducted a systematic review to assess the evidence for the impact of study design and setting on the interpretation of TB transmission using clustering derived from MIRU-VNTR strain typing—as has been shown using *IS6110* RFLP typing.

METHODS

Five electronic databases were searched (EMBASE, ISI Web of Science, CINAHL, Scopus and Medline (Ovid)) up to 20 October 2014. The search strategy combined the following terms with Boolean operators: Tuberculosis, strain typing, and transmission (see online supplementary appendix 1). The search was limited to studies using the standard MIRU-VNTR method,⁸ in humans only, and in English.

All titles and abstracts from each of the searches were examined. The full text of each paper was obtained and reviewed if the study reported MIRU-VNTR strain typing of *M. tuberculosis* complex isolates with at least 15 of the standardised 24 loci (Exact Tandem Repeat A, B, C, D, E; MIRU 2, 10, 16, 20, 23, 24, 26, 27, 39, 40; VNTR 424, 1955, 2163b, 2347, 2401, 3171, 3690, 4052, 4156).^{8 29 30}

Studies using fewer than 15 loci were not included because the level of discrimination is inadequate for epidemiological use (n=121).⁸ Studies that used loci different to the standardised 15 and 24 set were not included in the analysis in order to reduce the heterogeneity between studies (n=19). All publication types were included in this first screen to ensure that no relevant data were missed.

Reviews, letters, editorials, outbreaks or case reports (n=103) were excluded in the second screen. Studies that

used incomplete sampling (eg, random samples, studies using subsets of populations such as multidrug-resistant patients; n=47) and studies that had a sample size of less than 50 (n=4) were also excluded.

A reviewer (JM) extracted the following data items from all included studies using a form developed in Excel (Microsoft 2010): publication details (year, authors, study country), study details (study duration, loci typed, secondary typing method, study population, whether participant consent was required (a characteristic of the study design that was used as proxy for sampling fraction, assuming that where consent was required the sampling fraction was low)), the number of clustered and unique isolates and the covariates of interest: the maximum size of clusters; the proportion of clusters containing two cases; the proportion of the population that was culture positive; the proportion of culture positive isolates typed; risk factors for clustering; and the Hunter Gaston Discriminatory Index (HGDI)³¹. IA extracted data from 10% of the papers for external validity, disagreements were discussed and a consensus agreed on.

The main outcome measure—the proportion of TB isolates clustered by MIRU-VNTR strain typing—was calculated as the number of clustered isolates/number of clustered+unique isolates. Where there were uncertainties JM consulted with IA.

Authors were contacted if TB incidence rate was not reported. Where no response was received WHO country estimates of TB incidence for the study year were used.³² As so few studies reported the proportion coinfecting with TB/HIV, these estimates for the study country were taken from an European Union-wide survey and WHO country profiles.^{33 34} Owing to poor recording of the sampling fraction (the number of isolates typed/the total number of culture positive TB cases diagnosed during the study period (n=19)), whether the

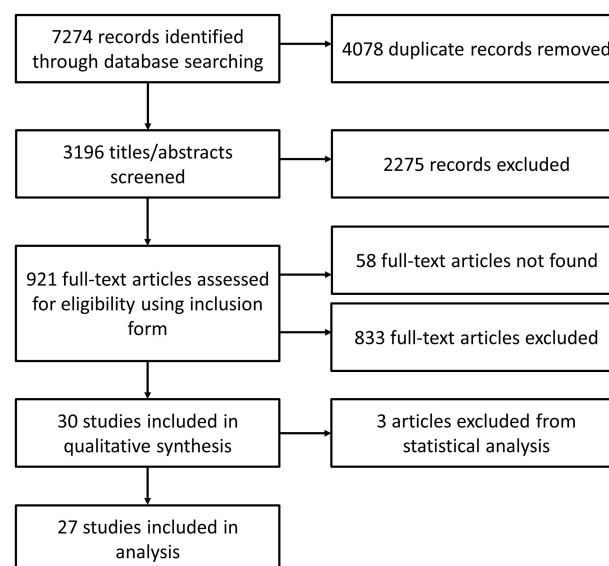


Figure 1 Results of systematic search, screening and data extraction.

Table 1 The study setting and design characteristics of the included articles

Reference	Study setting							Study design								
	Study area and country	TB incidence (per 100 000)	TB/HIV (per 100 000)‡	Previous TB treatment (%)	Pulmonary TB (%)	Maximum cluster size	Clusters of size 2 (%)	Study duration (months)	Study size (clustered +unique isolates)	Culture positive in study population (%)	Culture positive isolates typed (%)	Typing method§	Loci typed¶	Consent required	Risk of bias*	Clustering (%)†
51	New South Wales, Australia	6.7	0.2	0.0	63.7			36	1128			m24	N	no	low	20.1
40	Tabriz and Orumieh, Azarbaijan	26.0		5.2	87.0	5	81.8	12	156		94.5	m15	O	no	low	32.7
52	Brussels-Capital Region, Belgium	35.2	5.1	10.8		23	64.2	24	530	86.1	87.9	m24	N	no	low	29.6
53	Brussels-Capital Region, Belgium	35.2	5.1		100			39	802	81.8	84.7	m24s	N	no	low	28.8
54	Ontario, Canada	4.8	0.4			18	58.8	65	2016			m24s	N	no	low	23.1
37	Changping District, Beijing, China		0.3		100	0		30	318	31.5	94.6	m24	N	no	high	0.0
38	Croatia	19.0	0.1			45	48.3	36	1587			m15	N	no	high	62.8
55	Amhara region, Northwest Ethiopia		24.0	17.6	100	13		5	244			m24	N	yes	low	45.1
56	Finland	5.0	0.0			20		48	1048	75.4	99.4	m15s		no	low	33.9
57	Hamburg, Germany	12.7					45.5	12	154	78.2	91.1	m24s	N	no	low	22.1
45	Schleswig-Holstein, Germany	3.2	0.1			22	44.4	48	277			m24s	N	no	high	27.1
58	South West Ireland	15.3	3.3		82.7	12		36	171	79.5	96.1	m24s	N	no	low	27.5
59	South Tawara, Kiribati	370.0		4.1	100	25	55.6	24	73	45.4	98.6	m24s	N	yes	low	75.3
60	Netherlands	6.5	0.2				57.2	60	3978		100.1	m24	N	no	low	46.7
41	Kharkiv, Russia	94.0	3.8	63.3	100	10	50.0	3	98		100	m15	O	yes	high	31.6
61	Eastern province, Saudi Arabia	4.0			73.1	24	19.0	24	522			m24s	N	no	low	40.2
62	Singapore	40.5	1.2			21	48.0	24	1128	82.0	34.5	m24s	N	no	low	30.8
63	Slovenia	10.6	0.0			6		12	196	94.4	97.5	m24s	N	no	low	36.2
47	Almeria, Spain	26.0	6.0			8		27	281		81.9	m15	N	no	high	43.1
64	Sweden	4.8	0.1			10		36	406			m24s	N	no	low	21.2
65	Mubende, Uganda		86.0	31.1	87.8	11	70.0	6	67	21.5	90.5	m15s	N	yes	low	35.8
42	East Lancashire, UK	18.3	8.2			13	58.3	102	332	48.5	69.9	m15	O	no	low	42.8
39	UK		8.2		42.3	12	50.0	48	102	90.7	87.2	m15	O	no	low	30.4
66	London, UK	44.9	8.2					9	964	36.0	100	m24	N	no		37.0
43	Midlands, UK	15.0	8.2					48	4207	58.3	100	m15	O	no		61.2
44	Odessa and Nikolaev, Ukraine	80.4	3.9	34.2	100			4	225			m15	O	yes**	low	60.4
67	Hanoi, Vietnam	146.0	10.0	0.0	100			20	465	92.7	91.9	m15s	N	yes	low	55.3

*Risk of bias was assessed using the STROME-ID checklist. Studies scoring <20 were categorised as have a high risk of bias. See online supplementary appendix 2 for STROME-ID scores.

†The proportion of clustering was calculated as the number of clustered isolates/number of clustered+unique isolates.

‡Estimates from of the prevalence of TB/HIV coinfection in the study country.^{33 34}

§15=15 MIRU-VNTR loci (made up of the 'old 12' or 'new 12' defined in the footnote below), 24=24 MIRU-VNTR loci (ETR A, B, C, D, E; MIRU 2, 10, 16, 20, 23, 24, 26, 27, 39, 40; VNTR 424, 1955, 2163b, 2347, 2401, 3171, 3690, 4052, 4156), S=with Spoligotyping.

¶O=old 12 MIRU loci (MIRU 2, 4, 10, 16, 20, 23, 24, 26, 27,30, 31, 39, 40), N=new 12 MIRU loci (MIRU 10, 16, 26, 31, 40 +Mtub 04, 21, 39+ETR A C+QUB 11b, 26).

**11.3% did not consent to being part of the study. The other studies that required consent for isolates to be typed did not report the refusal rate.

ETR, Exact Tandem Repeat; TB, tuberculosis.

study required the consent of participants (yes/no) was included as a proxy for (low/high) sampling fraction. The risk of bias within each study was assessed using the STROME-ID checklist.³⁵

Data were analysed in Stata V.12. Where studies reported data from more than one set of loci, the method with the highest discriminatory value was included (ie, MIRU-VNTR 24 would be chosen over MIRU-VNTR 15, and MIRU-VNTR 15 plus Spoligotyping would be chosen over MIRU-VNTR 15 alone; n=8). This review was not concerned with summary measures of clustering, but factors that influenced clustering; therefore articles must have included at least one of the covariates. Continuous variables were transformed where the distribution was skewed. The proportion clustered was transformed using the Freeman Tukey transformation.³⁶ Study heterogeneity was assessed using a forest plot and the χ^2 test of heterogeneity. Univariable meta-regression analyses were carried out to determine the effect of the study design covariates on the proportion of clustered isolates. All covariates in the analysis were hypothesised to influence the proportion clustered a priori.

Sensitivity analyses were conducted to see the effect of removing studies reporting 0% clustering, with only extrapulmonary TB cases, only *Mycobacterium bovis* cases, studies using the 'old 12' MIRU loci as part of their 15 loci, and studies assessed as having a high likelihood of bias (STROME-ID score less than 20).

RESULTS

The search identified 7274 references resulting in 27 studies (25 journal articles and 2 conference abstracts) included after deduplication and title/abstract/full text screening (figure 1). The main characteristics of the

included studies are shown in table 1. Studies were published between 2007 and 2014 and the clustering reported varied from 0%³⁷ to 62.8%.³⁸ In all studies, clustered isolates were defined as having identical strain types based on the MIRU-VNTR loci typed, with or without Spoligotyping. Seventeen studies included isolates from newly diagnosed TB cases, three studies reported including isolates from new and chronic cases of TB, and seven did not report this information. In addition, 10 studies did not include repeat isolates from the same patient, one study included a repeat isolate from one patient and the remaining 17 did not report whether repeat isolates were included or not. Furthermore, four studies included isolates with missing loci in the cluster analysis, whereas four excluded isolates with missing loci and the remaining 20 did not report how they dealt with missing loci. The number of studies reporting each variable of interest is shown in table 2. STROME-ID scores can be found in online supplementary appendix 2.

A forest plot shows the spread of clustering reported by number of loci and additional typing method (figure 2). Significant heterogeneity was identified between the studies ($p<0.001$), suggesting that a meta-regression would be an appropriate analysis.

The univariable meta-regression shows evidence for the proportion of clustering to decrease as the number of MIRU-VNTR loci typed increased from 15 to 24 ($p=0.04$; table 3), accounting for 14% of the between study variation, and to increase when the study participants consented to being included in the study ($p=0.03$), accounting for 14% of the between study variation. The proportion of clustering increased as the TB incidence in the population increased ($p=0.007$, adjusted $R^2=26.7$). There was also evidence for the proportion of clustering

Table 2 The number of studies that reported the variables of interest

	Reported	Missing
Study setting		
TB incidence	8	15
TB/HIV coinfection	5	22
Previous TB treatment	9	18
Proportion pulmonary TB	14	13
Maximum cluster size	19	8
Percentage of clusters with 2 cases	14	13
Study design		
Study duration	27	0
Study size	27	0
Percentage of population that is culture positive	15	12
Percentage of culture positive typed	19	8
24 loci (compared to 15)	27	0
Repeat isolates	12	15
Missing loci	8	19
Double alleles	1	26
Consent required	6*	21
Epidemiological information	6	21

*Only one study reported the consent rate.
TB, tuberculosis.

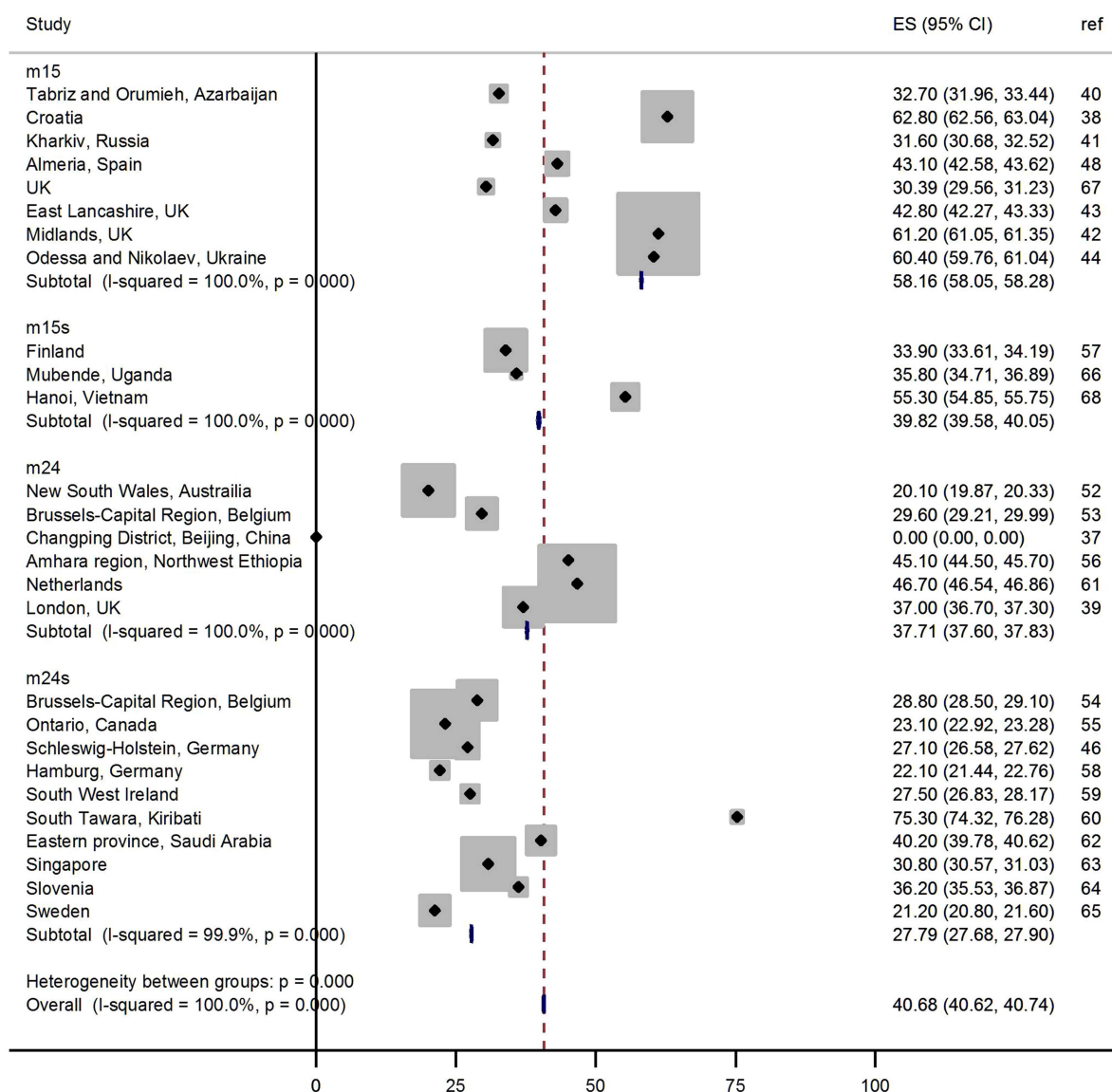


Figure 2 Forest plot showing the proportion of clustering reported in each study by the number of MIRU-VNTR loci typed. The number of loci typed is categorised into 15 loci (m15), 15 loci with Spoligotyping (m15 s), 24 loci (m24) and 24 loci with Spoligotyping (m24 s). The study reference is shown in the right hand column.

to increase as the maximum cluster size increased ($p=0.001$), accounting for 48% of between study variation. There was no evidence of the other study design or study setting variables significantly influencing the proportion clustered. Though non-significant ($p>0.05$), the TB/HIV coinfection rate in the population explained 2% of the between study variation. Too few studies included information on the proportion of clusters containing two cases, proportion of the study sample with previous TB or with pulmonary TB, so these could not be included in the analysis (table 2).

Sensitivity analyses to examine the effect of excluding studies reporting 0% clustering,³⁷ only *M. bovis* cases,³⁹ studies using the 'old 12' MIRU loci,³⁹⁻⁴⁴ and studies assessed as having a high risk of bias,^{37 38 45-47} did not generally change the results. The proportion of culture-positive TB in the population remained insignificant but

explained 2.6% of the between study variation when excluding 0% clustering ($p=0.278$ and adjusted $R^2=2.62$). Similarly, the proportion of culture-positive TB in the population remained insignificant but explained 2.6% of the between study variation when excluding studies with the highest risk of bias ($p=0.278$ and adjusted $R^2=2.62$). The number of loci typed became non-significant, but explained 9.6% and 10.5% of the between study variation when excluding studies using the 'old 12' loci and the highest risk of bias, respectively ($p=0.106$, adjusted $R^2=9.63$; $p=0.111$, adjusted $R^2=10.51$, respectively).

DISCUSSION

This review identified 27 studies that met the inclusion criteria. We illustrate that the interpretation of studies

Table 3 Univariable metaregression showing the coefficients for change in the proportion of clustering and the percentage of between-study variation explained by variables describing the study design and setting

	n	Coefficient*	CI	p Value	Adjusted R ² †
Study setting					
TB incidence	23	0.14	0.04 to 0.24	0.007	26.74
TB/HIV coinfection	23	0.04	−0.03 to 0.11	0.246	2.00
Maximum cluster size	19	0.20	0.09 to 0.30	0.001	48.20
Study design					
Study duration	27	−0.02	−0.09 to 0.06	0.677	−3.37
Percentage of population that is culture positive	15	0.34	−1.23 to 1.96	0.661	−5.92
Percentage of culture positive typed	19	0.22	−1.08 to 1.52	0.725	−5.41
Study size	27	0.03	−0.11 to 0.16	0.702	−3.31
24 loci (compared to 15)	27	−0.30	−0.59 to −0.01	0.04	13.58
Consent required	27	0.38	0.04 to 0.72	0.029	14.41

*Coefficients for the change in the proportion of clustering for each covariate. For example, for a one unit increase in maximum cluster size, the proportion of clustering increases by 0.2.

†The proportion of between-study variation explained by the univariate metaregression.
TB, tuberculosis.

using MIRU-VNTR to estimate clustering is subject to bias relating to study design and setting; however, there were insufficient data available to fully explore this impact.

As expected, we found that the proportion of clustering decreased with a greater number of MIRU-VNTR loci typed, with increasing TB incidence and with increasing maximum cluster size. We found that requiring consent to type patient isolates increased the proportion of clustering, which is not expected, given that the sampling fraction would be lower in these studies.

The other study design variables included in this analysis, such as study duration, did not significantly influence the proportion of isolates that were clustered, contrary to previous findings.¹² This is likely to be because of a lack of good quality evidence: of the 27 studies that met the inclusion criteria for the review, none reported all the variables of interest, reducing the power of the analysis and precluding multivariable metaregression (table 2). Importantly, key details of cluster analyses were not reported consistently across the studies, such as whether repeat isolates from the same patients were included, or typing profiles with missing loci were included, introducing new, unmeasured biases. In addition, the range of the variables may have been too limited to show any impact on clustering estimates. For example, the proportion of culture-positive isolates typed ranged from 34.5% to 100%, with 17 of the 19 studies reporting this variable from 81.9% to 100%. Furthermore, most of the studies (17/27=63%) were from low TB burden settings and therefore may be reflecting the rate at which imported cases have matching strain types by chance, rather than rates of recent transmission.

The sensitivity analysis suggested that, when excluding the studies with the greatest risk of bias, the culture-positivity in the population might explain a small amount of the between-study variation. This is consistent with estimates of the influence of sampling on the

proportion of clustering using IS6110 RFLP typing.⁴⁸ In the sensitivity analysis excluding studies that used the 'old 12' loci, the effect of the number of loci typed becomes non-significant. This is likely because studies using the 'old 12' accounted for six out of 10 studies reporting 15 loci, reducing the number of studies and the power of the model.

This study is a timely evaluation of the impact of study design on estimates of TB clustering using MIRU-VNTR strain typing because it has been incorporated into national typing services globally.^{23 49} The findings are relevant where strain typing is used to evaluate TB control systems across different settings because the proportion of clustering is influenced by the number of loci typed, the TB incidence and the maximum cluster size. Given that strain typing methods are advancing beyond MIRU-VNTR typing and that the application of whole genome sequencing to TB control and public health strategies has been demonstrated,^{9–11 50} it is important that the biases in the analysis of such methods are explored and compared. Understanding how to design and compare research studies for public health will greatly improve the benefit gained from newer technologies.

The strength of this meta-analysis was limited by the (lack of) detail reported by the included studies. This review has highlighted the need for better quality reporting in primary studies to enable future reviews to be more robust. Recently published standards for reporting of molecular epidemiology for infectious diseases should improve the quality of reporting.³⁵ This review is further limited by our inability to access 58 of the title/abstract screened articles for full text screening.

The use of TB strain typing as a public health tool in TB control programmes is increasing globally. We have identified a lack of good quality studies that can contribute to our understanding in interpreting the molecular typing of TB. We have also shown that the proportion of clustering derived from MIRU-VNTR typing is influenced by the number of loci typed, whether consent is

required to type isolates, TB incidence in the study setting and the maximum cluster size, highlighting these as important considerations in the design and interpretation of future studies.

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