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

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Abstract

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

Data sources: Medline, Embase, CINHAL, Web of Science and Scopus were searched for articles published before November 2012

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 14 eligible articles reporting clustering between 22.1% and 61.2%. The proportion of culture positive isolates and the number of MIRU-VNTR loci typed explained 49% and 34% of the 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.

Strengths and Limitations of Study

- This 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.
- There were insufficient data available to fully explore the impact of study design and setting on estimates of clustering.

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 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* (IS*6110*) 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 IS*6110* 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 IS*6110* 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 IS*6110* RFLP, MIRU-VNTR does not have to exclude isolates with a low IS*6110* 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 IS*6110* 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 IS*6110* RFLP typing.

Methods

Five electronic databases were searched (EMBASE, ISI Web of Science, CINHAL, Scopus and Medline (Ovid)) up to 1 November 2012. The search strategy combined the following terms with Boolean operators: Tuberculosis, strain typing, and transmission. 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 [8,29,30].

Studies using fewer than 15 loci were not included because the level of discrimination is inadequate for epidemiological use (n=97) [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=11). 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=99) were excluded in the second screen. Studies that used incomplete sampling (e.g. random samples, studies using subsets of populations such as MDR patients) (n=30) and studies that had a sample size of less than 50 (n=2) were also excluded.

A reviewer 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), the proportion of total TB isolates clustered by MIRU-VNTR strain typing, and the covariates of interest: the number of clustered and unique isolates; the maximum size of clusters; the proportion of clusters containing two cases; the prevalence of culture-positivity among TB patients included in the study; the proportion of culture positive isolates typed; risk factors for clustering; and the Hunter Gaston Discriminatory Index (HGDI) [31]).

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].

Data were analysed in Stata 12. Where studies reported data from more than one set of loci, the method with the highest discriminatory value was included (i.e. 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=5). 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 [33]. 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*.

Results

The search identified 5607 references resulting in 12 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 2011 and the clustering reported varied from 22.1% [34] to 61.2% [35].

The univariable meta-regression shows evidence for the proportion of clustering to decrease as the prevalence of culture-positivity among TB patients included in the study increases (p=0.03; Table 2), accounting for 49% of the between study variation. There was also evidence for the proportion of clustering to decrease as the number of MIRU-VNTR loci typed increased from 15 to 24 (p=0.02), explaining 34% of the 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 size of the study and the maximum cluster size explained 15% and 27% of the between study variation, respectively.

Discussion

This review identified 14 studies that met the inclusion criteria. We illustrate that the interpretation of studies using MIRU-VNTR to estimate clustering is subject to bias relating to study design; however, there were insufficient data available to fully explore the impact of study design and setting on estimates of clustering.

As expected, we found that the proportion of clustering decreased with a greater number of MIRU-VNTR loci typed. Our finding that the prevalence of culture-positivity among TB patients included in the study influences the estimates of transmission within a population is counterintuitive and not consistent with estimates of the influence of sampling on the proportion of clustering using *IS*6110 RFLP typing [36]. This may reflect the relationship between TB burden and resource poor/rich

settings and the consequent availability of culture diagnostic laboratory services; i.e. in resource poor settings where there is a high burden of TB (and, therefore, high rates of clustering) the prevalence of culture positive TB cases is low. The finding may also be due to chance, with only 8 studies included in the analysis of this variable.

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: only 14 studies met the inclusion criteria for the review and of those only three reported all the variables of interest, reducing the power of the analysis and precluding multivariable meta-regression. 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 had a narrow range from 81.9% to 100%. Furthermore, most of the studies 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.

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 [23,37]. 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 prevalence of culture positive TB cases in the study setting. 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 [9–11,38], 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.

This review has highlighted the need for better quality reporting in primary studies to enable future reviews to be more robust. A lack of standards for the molecular epidemiology of infectious diseases may explain the poor quality of reporting; this field would benefit from the introduction of such standards (STROBE-ID, submitted).

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-VTNR typing is influenced by the number of loci typed and the prevalence of culture-positivity among TB patients included in the study, highlighting these as important considerations in the design and interpretation of future studies.

Conflict of interest

Nothing to declare.

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Author contributions

All authors made substantial contributions to the conception and design of the review, and the analysis and interpretation of data. JM drafted the article and PS, IA, TM and TC revised it critically for important intellectual content. All authors approved the final version for publication.

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Ethics

Ethical approval was not required as this review analyses data that is in the public domain.

Data sharing

No additional data are available

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Tables

Table 1: Studies included in the analysis

Reference	Author	Country	Studysite [a]	Method [b]	Loci [c]	Study duration (months)	Clustered + unique isolates	TB incidence (per 100,000)	TB/HIV co-infection [d]	Prevalence of culture positivity	% culture positive typed	No. clusters	Max cluster size	HGDI	Proportion clustering	Recent transmission (%)
[39]	Asgharzadeh, M	Azerbaijan	r	15	o	12	156	26.0		94.6	98.7	22	5	0.9966	32.7	18.6
[40]	Allix-Beguec, C	Belgium	r	24	n	24	530	35.2	5.1	86.1	87.9	53	23		29.6	19.6
[41]	Allix-Beguec, C	Belgium	r	24,S	n	39	802	35.2	5.1	81.8	84.7	82			28.8	19.6
[34]	Oelemann, M	Germany	ci	24,S	n	12	154	12.7			100	11			22.1	14.9
[42]	Roetzer, A	Germany	r	24,S	n	48	277	3.2	0.09		100	18	22		27.1	20.6
[43]	Ojo, OO	Ireland	r	24,S	n	36	171	15.3	3.3	79.5	96.1	15	12	0.9996	27.5	18.7
[44]	Dymova, MA	Russia	r	15	o	3	98	94.0	3.8		100	8		0.9900	31.6	23.5
[45]	Bidovec-Stojkovic, U	Slovenia	co	24,S	n	12	196	10.6	0.04		100	29	6	0.9965	36.2	21.4
[46]	Alonso-Rodriguez, N	Spain	r	15	n	27	281	26.0	6		81.9		8		43.1	24.4
[35]	Evans, J	UK	r	15	o	48	4207	15.0	8.2	58.3	100	439			61.2	50.8
[47]	Hamblion, E	UK	r	24	n	9	964	44.9	8.2		100				37.0	
[48]	Mandal, S	UK	co	15	o	48	102		8.2	90.7	87.2	8	12		30.4	22.6
[49]	Sails, A	UK	r	15	o	102	332	18.3	8.2	33.9	100	42	13		42.8	30.1
[50]	Nikolayevsky, V	Ukraine	r	15	o	4	225	80.4	3.9	39.2	97.4	31		0.9700	60.4	46.7

[a] ci=city, r=region, co=country

[b] 15=15 MIRU-VNTR loci, 24=24 MIRU-VNTR loci, S=with Spoligotyping

[c] 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)

[d] estimates from the literature of the prevalence of TB/HIV co-infection reported in the study area

Table 2: 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.

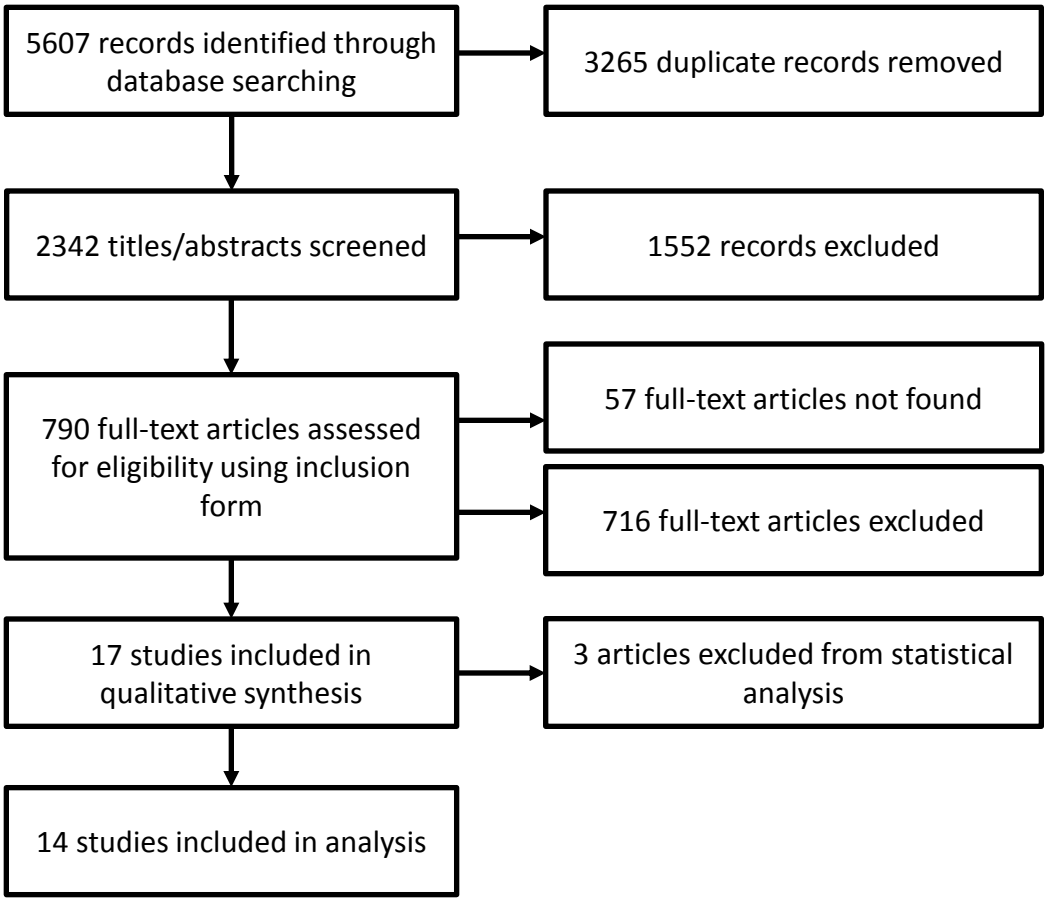
	n	Coefficient [π]	CI	p	Adj R^{2} [¥]
Study design					
Study duration (months)	14	0.003	-0.063, 0.069	0.919	-8.47
Prevalence of culture positivity	8	-0.913	-1.732, -0.094	0.034	49.36
% culture positive typed	14	0.161	-0.731, 1.053	0.701	-6.99
Study size	14	-4.462	-10.000, 1.076	0.105	14.89
Number of loci (ref 15 loci)					
24 loci	14	-0.282	-0.519, -0.045	0.023	34.1
Study setting					
TB incidence	13	0.082	-0.097, 0.22	0.334	0.04
TB/HIV co-infection	12	0.088	-0.087, 0.263	0.288	3.28
Maximum cluster size	9	0.137	-0.035, 0.309	0.101	26.91
% clusters with 2 cases	7	0.004	-0.007, 0.016	0.396	-2.39

[π] Coefficients for the change in the proportion of clustering for each covariate. E.g. for a one-month increase in study duration, the proportion of clustering increases by 0.003.

[¥] The proportion of between-study variation explained by the univariate metaregression.

Figure Caption

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


5607 records identified through database searching
3265 duplicate records removed
2342 titles/abstracts screened
1552 records excluded
790 full-text articles assessed for eligibility using inclusion form
57 full-text articles not found
716 full-text articles excluded
17 studies included in qualitative synthesis
3 articles excluded from statistical analysis
14 studies included in analysis

Appendix: Medline/Embase search strategy

1. (tubercle adj3 (bacillus or bacilli)).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

2. ((mycobacterium or mycobacteria) adj3 (bovis or africanum or microti or canetti)).mp.

3. exp tuberculosis/ or mycobacterium tuberculosis/ or tuberculosis.mp. or tb.mp. or Mtb.mp. or "M tuberculosis complex".mp.

4. or/1-3

5. Minisatellite Repeats/ or Genotype/ or Interspersed Repetitive Sequences/ or DNA Fingerprinting/ or Bacterial Typing Techniques/

6. "miru".mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

7. "vntr".mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

8. (miru adj3 vntr).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

9. (mycobacterial adj3 interspersed adj3 repetitive adj3 units).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

10. (dna adj3 fingerprinting).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

11. ((strain adj3 type) or (strain adj3 typing) or (strain adj3 types)).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

12. ((molecular adj3 typing) or (molecular adj3 strain adj3 typ*)).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

13. (genotype or genotyping or genotypes).ti,ab.

14. (minisatellite adj3 repeat*).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

15. molecular epidemiology/mt or (molecular adj3 epidemiology).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

16. or/5-15

17. exp disease outbreaks/ or (outbreak adj3 analysis).mp. or (outbreak adj3 investigation).mp. or (outbreak adj3 management).mp. or (tuberculosis adj3 outbreak).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

18. exp contact tracing/ or (contact adj3 tracing).mp. or (contact* adj3 traced).mp. or (contact adj3 screen*).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

19. exp case management/ or (case adj3 management).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

20. exp Risk Factors/

21. (risk adj3 factor*).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

22. exp Epidemiologic Factors/

23. infectious disease transmission.mp. or exp Disease Transmission, Infectious/

24. exp case management/ or (case adj3 management).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

25. program evaluation/ or evaluation studies as topic/ or (program adj3 evaluation).mp. or (programme adj3 evaluation).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

26. public health practice/ or (public adj3 health).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

27. ((tuberculosis adj3 control) or (tb adj3 control)).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

28. (molecular adj3 surveillance).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

29. exp cluster analysis/ or (cluster* adj3 rate*).mp. or (cluster* adj3 growth).mp. or (cluster* adj3 analysis).mp. or (cluster adj3 investigation).mp. or (proportion adj3 cluster*).mp. or (molecular adj3 cluster*).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

30. ((recent adj3 transmission) or (transmission adj3 event*) or (transmission adj3 rate*) or (chain adj3 transmission) or (transmission adj3 setting*)).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

31. or/17-30

32. 4 and 16

33. 32 and 31

34. limit 33 to yr="1998-Current"

35. limit 34 to english language

36. animals/

37. humans/

38. 36 not 37

39. 35 not 38

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	n/a
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	14
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	4
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	4
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	5
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	5

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	4
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	5
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	5
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	12
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	12
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	12
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	14
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	12
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	14
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	5
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	6
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	6
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	7

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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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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 TB transmission using clustering derived from Mycobacterial Interspersed Repetitive Units – Variable Number Tandem Repeats (MIRU-VNTR) strain typing.

Data sources: Medline, Embase, CINHAL, 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%, 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.

Strengths and Limitations of Study

- This 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.
- 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.

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 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* (IS*6110*) 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 IS*6110* 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 IS*6110* 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 IS*6110* RFLP, MIRU-VNTR does not have to exclude isolates with a low IS*6110* 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 IS*6110* 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 IS*6110* RFLP typing.

Methods

Five electronic databases were searched (EMBASE, ISI Web of Science, CINHAL, Scopus and Medline (Ovid)) up to 20th October 2014. The search strategy combined the following terms with Boolean operators: Tuberculosis, strain typing, and transmission. 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 (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) [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 (e.g. 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 upon.

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 coinfected with TB/HIV, these estimates for the study country were taken from an EU-wide survey and WHO country profiles.[33,34] Due 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 study required the consent of participants (yes/no) was included as a proxy for (high/low) sampling fraction. The risk of bias within each study was assessed using the STROME-ID checklist. [35]

Data were analysed in Stata 12. Where studies reported data from more than one set of loci, the method with the highest discriminatory value was included (i.e. 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 chi^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 extra-pulmonary TB cases, only *M.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. 17 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, ten 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.

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 ($p=0.007$, Adj $R^2 = 26.7$). There was also evidence for the proportion of clustering 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, and the proportion of the population with culture positive 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,[37,45–48] 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 Adj $R^2=2.62$). 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 Adj $R^2=2.62$). 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, Adj R^2=9.63; p=0.111, Adj R^2=10.51, respectively).

Discussion

This review identified 27 studies that met the inclusion criteria. We illustrate that the interpretation of studies 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 reduced the proportion of clustering, which is 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 meta-regression (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 counterintuitive and not consistent with estimates of the influence of sampling on the proportion of clustering using *IS*6110 RFLP typing [49]. This may reflect the relationship between TB burden and resource poor/rich settings and the consequent availability of culture diagnostic laboratory services; i.e. in resource poor settings where there is a high burden of TB (and, therefore, high rates of clustering) the prevalence of culture positive TB cases is low. The finding may also be due to chance, with only 14 studies included in the analysis of this variable. 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 ten 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 [23,50]. 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 [9–11,51], 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 (a 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-VTNR 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.

Conflict of interest

Nothing to declare.

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Author contributions

All authors made substantial contributions to the conception and design of the review, and the analysis and interpretation of data. JM drafted the article and PS, IA, TM and TC revised it critically for important intellectual content. All authors approved the final version for publication.

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Ethics

Ethical approval was not required as this review analyses data that is in the public domain.

Data sharing

No additional data are available

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Tables

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

Ref	Study setting							Study design							Risk of bias [d]	Clustering (%)[e]
	Study area and country	TB incidence (per 100,000)	TB/HIV (per 100,000)[a]	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 [b]	Loci typed [c]	Consent required		
[52]	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
[53]	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
[54]	Brussels-Capital Region, Belgium	35.2	5.1	.	100	.	.	39	802	81.8	84.7	m24s	N	no	low	28.8
[55]	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
[56]	Amhara region, Northwest Ethiopia	.	24.0	17.6	100	13	.	5	244	.	.	m24	N	yes	low	45.1
[57]	Finland	5.0	0.0	.	.	20	.	48	1048	75.4	99.4	m15s	.	no	low	33.9
[58]	Hamburg, Germany	12.7	.	.	.	.	45.5	12	154	78.2	91.1	m24s	N	no	low	22.1
[46]	Schleswig-Holstein, Germany	3.2	0.1	.	.	22	44.4	48	277	.	.	m24s	N	no	high	27.1
[59]	South West Ireland	15.3	3.3	.	82.7	12	.	36	171	79.5	96.1	m24s	N	no	low	27.5
[60]	South Tawara, Kiribati	370.0	.	4.1	100	25	55.6	24	73	45.4	98.6	m24s	N	yes	low	75.3
[61]	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
[62]	Eastern province, Saudi Arabia	4.0	.	.	73.1	24	19.0	24	522	.	.	m24s	N	no	low	40.2

[63]	Singapore	40.5	1.2	.	.	21	48.0	24	1128	82.0	34.5	m24s	N	no	low	30.8
[64]	Slovenia	10.6	0.0	.	.	6	.	12	196	94.4	97.5	m24s	N	no	low	36.2
[48]	Almeria, Spain	26.0	6.0	.	.	8	.	27	281	.	81.9	m15	N	no	high	43.1
[65]	Sweden	4.8	0.1	.	.	10	.	36	406	.	.	m24s	N	no	low	21.2
[66]	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
[67]	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[f]	low	60.4
[68]	Hanoi, Vietnam	146.0	10.0	0.0	100	.	.	20	465	92.7	91.9	m15s	N	yes	low	55.3

[a] Estimates from of the prevalence of TB/HIV co-infection in the study country [33,34]

[b] 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

[c] 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)

[d] Risk of bias was assessed using the STROME-ID checklist. Studies scoring <20 were categorised as have a high risk of bias

[e] The proportion of clustering was calculated as the number of clustered isolates/number of clustered + unique isolates

[f] 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

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

	Reported	Missing
Study setting		
TB incidence	8	15
TB/HIV co-infection	5	22
Previous TB treatment	9	18
Proportion pulmonary TB	14	13
Maximum cluster size	19	8
% clusters with 2 cases	14	13
Study design		
Study duration	27	0
Study size	27	0
% population that is culture positive	15	12
% 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[a]	21
Epidemiological information	6	21

[a] Only one study reported the consent rate

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 [a]	CI	p	Adj R^{2} [b]
Study setting					
TB incidence	23	0.14	0.04-0.24	0.007	26.74
TB/HIV co-infection	23	0.04	-0.03-0.11	0.246	2.00
Maximum cluster size	19	0.20	0.09-0.30	0.001	48.20
Study design					
Study duration	27	-0.02	-0.09-0.06	0.677	-3.37
% population that is culture positive	15	0.34	-1.23-1.96	0.661	-5.92
% culture positive typed	19	0.22	-1.08-1.52	0.725	-5.41
Study size	27	0.03	-0.11-0.16	0.702	-3.31
24 loci (compared to 15)	27	-0.30	-0.59--0.01	0.04	13.58
Consent required	27	0.38	0.04-0.72	0.029	14.41

[a] Coefficients for the change in the proportion of clustering for each covariate. E.g. for a one-month increase in study duration, the proportion of clustering increases by 0.003.

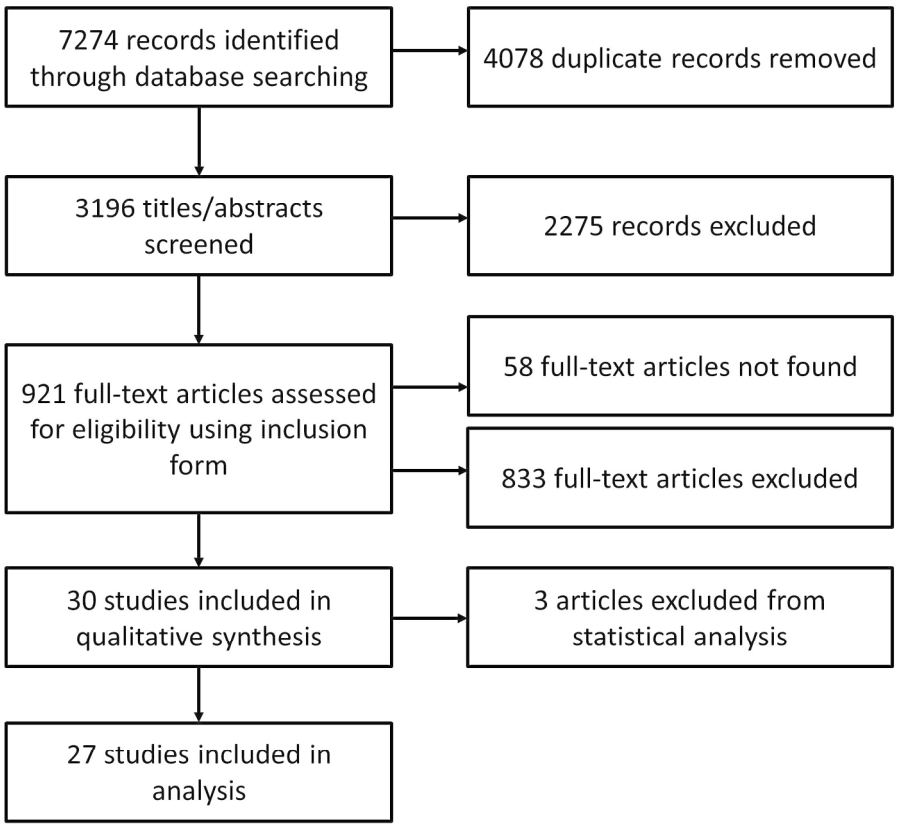
[b] The proportion of between-study variation explained by the univariate meta-regression.

Figure Caption

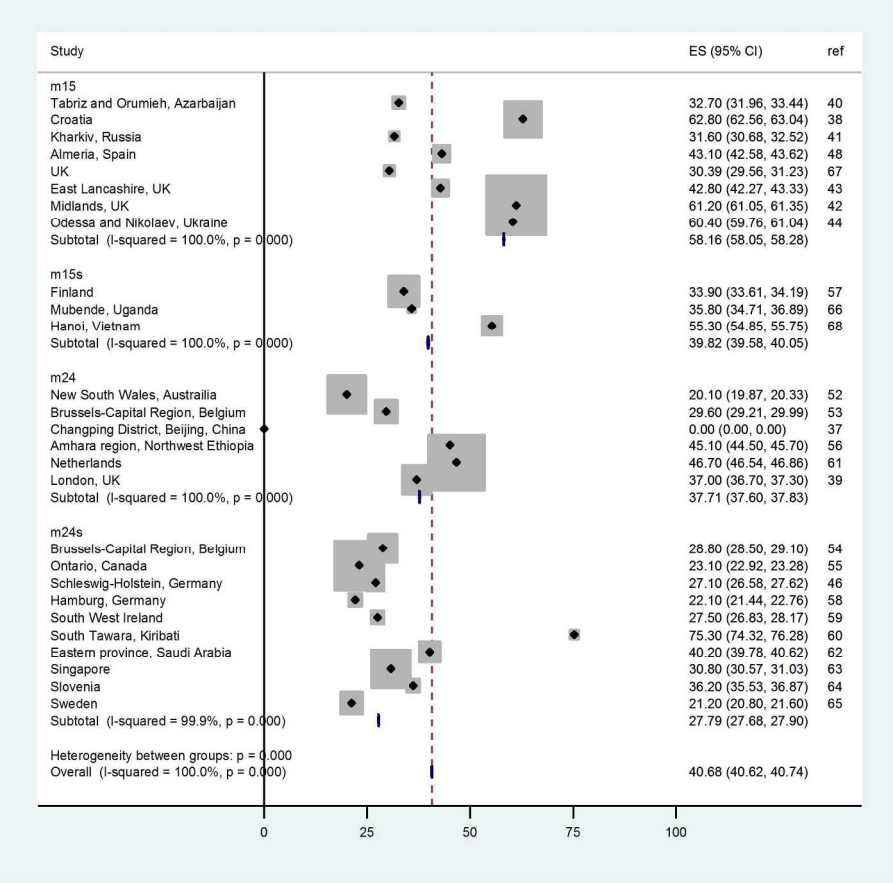
Figure 1: Results of systematic search, screening and data extraction.

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 (m15s), 24 loci (m24) and 24 loci with Spoligotyping (m24s). The study reference is shown in the right hand column.



190x254mm (300 x 300 DPI)



190x254mm (300 x 300 DPI)

Appendix 1: Medline/Embase search strategy

1. (tubercle adj3 (bacillus or bacilli)).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

2. ((mycobacterium or mycobacteria) adj3 (bovis or africanum or microti or canetti)).mp.

3. exp tuberculosis/ or mycobacterium tuberculosis/ or tuberculosis.mp. or tb.mp. or Mtb.mp. or "M tuberculosis complex".mp.

4. or/1-3

5. Minisatellite Repeats/ or Genotype/ or Interspersed Repetitive Sequences/ or DNA Fingerprinting/ or Bacterial Typing Techniques/

6. "miru".mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

7. "vntr".mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

8. (miru adj3 vntr).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

9. (mycobacterial adj3 interspersed adj3 repetitive adj3 units).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

10. (dna adj3 fingerprinting).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

11. ((strain adj3 type) or (strain adj3 typing) or (strain adj3 types)).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

12. ((molecular adj3 typing) or (molecular adj3 strain adj3 typ*)).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

13. (genotype or genotyping or genotypes).ti,ab.

14. (minisatellite adj3 repeat*).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

15. molecular epidemiology/mt or (molecular adj3 epidemiology).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

16. or/5-15

17. exp disease outbreaks/ or (outbreak adj3 analysis).mp. or (outbreak adj3 investigation).mp. or (outbreak adj3 management).mp. or (tuberculosis adj3 outbreak).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

18. exp contact tracing/ or (contact adj3 tracing).mp. or (contact* adj3 traced).mp. or (contact adj3 screen*).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

19. exp case management/ or (case adj3 management).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

20. exp Risk Factors/

21. (risk adj3 factor*).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

22. exp Epidemiologic Factors/

23. infectious disease transmission.mp. or exp Disease Transmission, Infectious/

24. exp case management/ or (case adj3 management).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

25. program evaluation/ or evaluation studies as topic/ or (program adj3 evaluation).mp. or (programme adj3 evaluation).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

26. public health practice/ or (public adj3 health).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

27. ((tuberculosis adj3 control) or (tb adj3 control)).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

28. (molecular adj3 surveillance).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

29. exp cluster analysis/ or (cluster* adj3 rate*).mp. or (cluster* adj3 growth).mp. or (cluster* adj3 analysis).mp. or (cluster adj3 investigation).mp. or (proportion adj3 cluster*).mp. or (molecular adj3 cluster*).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

30. ((recent adj3 transmission) or (transmission adj3 event*) or (transmission adj3 rate*) or (chain adj3 transmission) or (transmission adj3 setting*)).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

31. or/17-30

32. 4 and 16

33. 32 and 31

34. limit 33 to yr="1998-Current"

35. limit 34 to english language

36. animals/

37. humans/

38. 36 not 37

39. 35 not 38

Appendix 2: STROME-ID scores for the included studies

Author	STROME-ID score[a]
Aleksic, E	24
Alliex-Beguec, C	32
Allix-Beguec, C	25
Alonso-Rodriguez, N	18
Asgharzadeh, M	28
Bidovec-Stojkovic, U	31
De Beer, JL	30
Dymova, MA	19
Evans, J	[b]
Grujav, U	32
Guang-ming, DAI	19
Hamblion, E	[b]
Hang, NTHL	31
Jonsson, J	22
Lim, LKY	30
Mandal, S	32
Muwonge, A	25
Nikolayevsky, V	23
Oelemann, M	34
Ojo, OO	36
Roetzer, A	16
Sails, A	23
Smit, PW	29
Tessema, B	26
Tuite, AR	31
Varghese, B	23
Zmak, L	19

[a]Individual studies score 1 for each element of checklist they had address

[b]Conference abstracts

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	n/a
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	appendix
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	4
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	5
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	5
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	5

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	5
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	5
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	15
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	15
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Figure 2
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	18
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	15
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	18
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	7
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	7-8
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	8
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	8

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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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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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 TB transmission using clustering derived from Mycobacterial Interspersed Repetitive Units – Variable Number Tandem Repeats (MIRU-VNTR) strain typing.

Data sources: Medline, Embase, CINHAL, 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.

Strengths and Limitations of Study

- This 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.
- 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.

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 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* (IS*6110*) 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 IS*6110* 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 IS*6110* 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 IS*6110* RFLP, MIRU-VNTR does not have to exclude isolates with a low IS*6110* 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 IS*6110* 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 IS*6110* RFLP typing.

Methods

Five electronic databases were searched (EMBASE, ISI Web of Science, CINHAL, Scopus and Medline (Ovid)) up to 20th October 2014. The search strategy combined the following terms with Boolean operators: Tuberculosis, strain typing, and transmission (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 (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) [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 (e.g. 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 upon.

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 coinfected with TB/HIV, these estimates for the study country were taken from an EU-wide survey and WHO country profiles.[33,34] Due 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 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 12. Where studies reported data from more than one set of loci, the method with the highest discriminatory value was included (i.e. 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 chi^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 extra-pulmonary TB cases, only *M.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. 17 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, ten 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 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 ($p=0.007$, Adj $R^2 = 26.7$). There was also evidence for the proportion of clustering 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,[37,45–48] 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 Adj $R^2=2.62$). 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 Adj $R^2=2.62$). 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, Adj R^2=9.63; p=0.111, Adj R^2=10.51, respectively).

Discussion

This review identified 27 studies that met the inclusion criteria. We illustrate that the interpretation of studies 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 meta-regression (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 *IS*6110 RFLP typing [49]. 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 ten 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 [23,50]. 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 [9–11,51], 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 (a 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-VTNR 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.

Conflict of interest

Nothing to declare.

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Author contributions

All authors made substantial contributions to the conception and design of the review, and the analysis and interpretation of data. JM drafted the article and PS, IA, TM and TC revised it critically for important intellectual content. All authors approved the final version for publication.

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Ethics

Ethical approval was not required as this review analyses data that is in the public domain.

Data sharing

No additional data are available

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Tables

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

Ref	Study setting							Study design							Risk of bias [d]	Clustering (%)[e]
	Study area and country	TB incidence (per 100,000)	TB/HIV (per 100,000)[a]	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 [b]	Loci typed [c]	Consent required		
[52]	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
[53]	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
[54]	Brussels-Capital Region, Belgium	35.2	5.1	.	100	.	.	39	802	81.8	84.7	m24s	N	no	low	28.8
[55]	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
[56]	Amhara region, Northwest Ethiopia	.	24.0	17.6	100	13	.	5	244	.	.	m24	N	yes	low	45.1
[57]	Finland	5.0	0.0	.	.	20	.	48	1048	75.4	99.4	m15s	.	no	low	33.9
[58]	Hamburg, Germany	12.7	.	.	.	.	45.5	12	154	78.2	91.1	m24s	N	no	low	22.1
[46]	Schleswig-Holstein, Germany	3.2	0.1	.	.	22	44.4	48	277	.	.	m24s	N	no	high	27.1
[59]	South West Ireland	15.3	3.3	.	82.7	12	.	36	171	79.5	96.1	m24s	N	no	low	27.5
[60]	South Tawara, Kiribati	370.0	.	4.1	100	25	55.6	24	73	45.4	98.6	m24s	N	yes	low	75.3
[61]	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
[62]	Eastern province, Saudi Arabia	4.0	.	.	73.1	24	19.0	24	522	.	.	m24s	N	no	low	40.2

[63]	Singapore	40.5	1.2	.	.	21	48.0	24	1128	82.0	34.5	m24s	N	no	low	30.8
[64]	Slovenia	10.6	0.0	.	.	6	.	12	196	94.4	97.5	m24s	N	no	low	36.2
[48]	Almeria, Spain	26.0	6.0	.	.	8	.	27	281	.	81.9	m15	N	no	high	43.1
[65]	Sweden	4.8	0.1	.	.	10	.	36	406	.	.	m24s	N	no	low	21.2
[66]	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
[67]	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[f]	low	60.4
[68]	Hanoi, Vietnam	146.0	10.0	0.0	100	.	.	20	465	92.7	91.9	m15s	N	yes	low	55.3

[a] Estimates from of the prevalence of TB/HIV co-infection in the study country [33,34]

[b] 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

[c] 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)

[d] Risk of bias was assessed using the STROME-ID checklist. Studies scoring <20 were categorised as have a high risk of bias. See Appendix 2 for STROME-ID scores

[e] The proportion of clustering was calculated as the number of clustered isolates/number of clustered + unique isolates

[f] 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

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

	Reported	Missing
Study setting		
TB incidence	8	15
TB/HIV co-infection	5	22
Previous TB treatment	9	18
Proportion pulmonary TB	14	13
Maximum cluster size	19	8
% clusters with 2 cases	14	13
Study design		
Study duration	27	0
Study size	27	0
% population that is culture positive	15	12
% 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[a]	21
Epidemiological information	6	21

[a] Only one study reported the consent rate

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 [a]	CI	p	Adj R^{2} [b]
Study setting					
TB incidence	23	0.14	0.04-0.24	0.007	26.74
TB/HIV co-infection	23	0.04	-0.03-0.11	0.246	2.00
Maximum cluster size	19	0.20	0.09-0.30	0.001	48.20
Study design					
Study duration	27	-0.02	-0.09-0.06	0.677	-3.37
% population that is culture positive	15	0.34	-1.23-1.96	0.661	-5.92
% culture positive typed	19	0.22	-1.08-1.52	0.725	-5.41
Study size	27	0.03	-0.11-0.16	0.702	-3.31
24 loci (compared to 15)	27	-0.30	-0.59--0.01	0.04	13.58
Consent required	27	0.38	0.04-0.72	0.029	14.41

[a] Coefficients for the change in the proportion of clustering for each covariate. E.g. for a one unit increase in maximum cluster size, the proportion of clustering increases by 0.2.

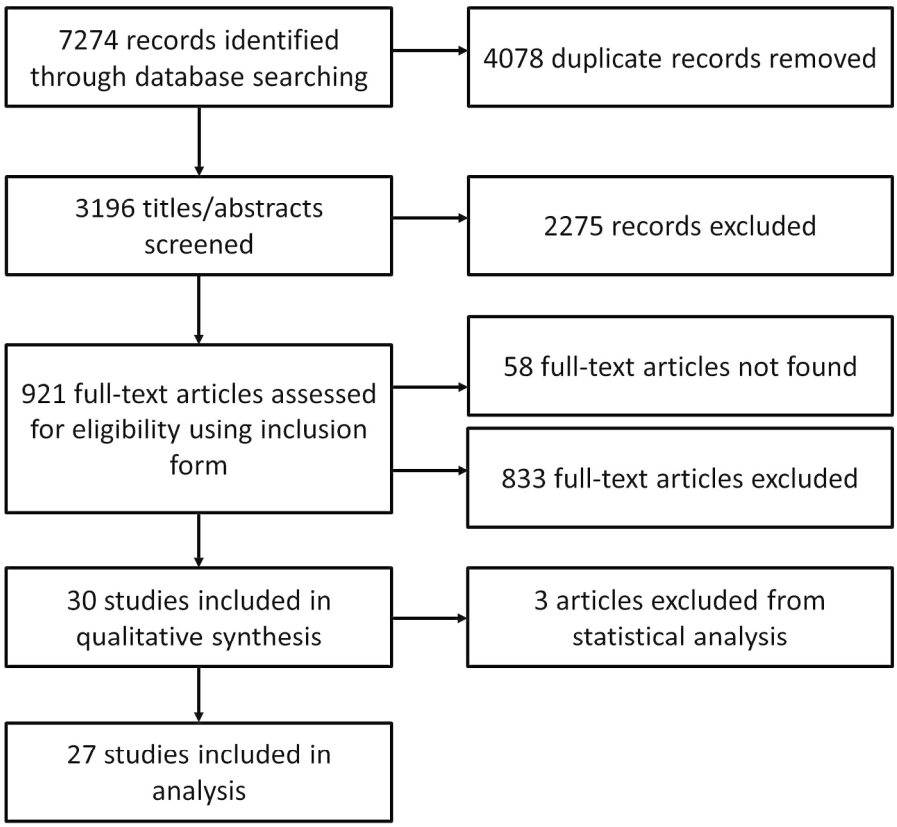
[b] The proportion of between-study variation explained by the univariate meta-regression.

Figure Caption

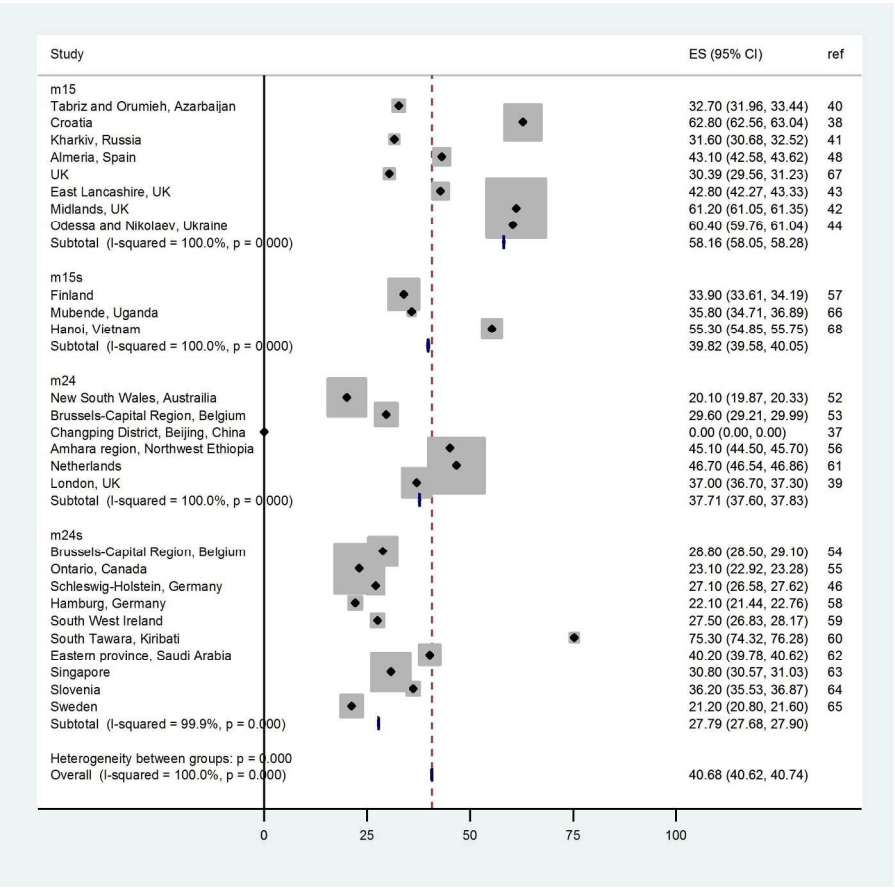
Figure 1: Results of systematic search, screening and data extraction.

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 (m15s), 24 loci (m24) and 24 loci with Spoligotyping (m24s). The study reference is shown in the right hand column.



190x254mm (300 x 300 DPI)



190x254mm (300 x 300 DPI)

Appendix 1: Medline/Embase search strategy

1. (tubercle adj3 (bacillus or bacilli)).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

2. ((mycobacterium or mycobacteria) adj3 (bovis or africanum or microti or canetti)).mp.

3. exp tuberculosis/ or mycobacterium tuberculosis/ or tuberculosis.mp. or tb.mp. or Mtb.mp. or "M tuberculosis complex".mp.

4. or/1-3

5. Minisatellite Repeats/ or Genotype/ or Interspersed Repetitive Sequences/ or DNA Fingerprinting/ or Bacterial Typing Techniques/

6. "miru".mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

7. "vntr".mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

8. (miru adj3 vntr).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

9. (mycobacterial adj3 interspersed adj3 repetitive adj3 units).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

10. (dna adj3 fingerprinting).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

11. ((strain adj3 type) or (strain adj3 typing) or (strain adj3 types)).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

12. ((molecular adj3 typing) or (molecular adj3 strain adj3 typ*)).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

13. (genotype or genotyping or genotypes).ti,ab.

14. (minisatellite adj3 repeat*).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

15. molecular epidemiology/mt or (molecular adj3 epidemiology).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

16. or/5-15

17. exp disease outbreaks/ or (outbreak adj3 analysis).mp. or (outbreak adj3 investigation).mp. or (outbreak adj3 management).mp. or (tuberculosis adj3 outbreak).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

18. exp contact tracing/ or (contact adj3 tracing).mp. or (contact* adj3 traced).mp. or (contact adj3 screen*).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

19. exp case management/ or (case adj3 management).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

20. exp Risk Factors/

21. (risk adj3 factor*).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

22. exp Epidemiologic Factors/

23. infectious disease transmission.mp. or exp Disease Transmission, Infectious/

24. exp case management/ or (case adj3 management).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

25. program evaluation/ or evaluation studies as topic/ or (program adj3 evaluation).mp. or (programme adj3 evaluation).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

26. public health practice/ or (public adj3 health).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

27. ((tuberculosis adj3 control) or (tb adj3 control)).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

28. (molecular adj3 surveillance).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

29. exp cluster analysis/ or (cluster* adj3 rate*).mp. or (cluster* adj3 growth).mp. or (cluster* adj3 analysis).mp. or (cluster adj3 investigation).mp. or (proportion adj3 cluster*).mp. or (molecular adj3 cluster*).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

30. ((recent adj3 transmission) or (transmission adj3 event*) or (transmission adj3 rate*) or (chain adj3 transmission) or (transmission adj3 setting*)).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]

31. or/17-30

32. 4 and 16

33. 32 and 31

34. limit 33 to yr="1998-Current"

35. limit 34 to english language

36. animals/

37. humans/

38. 36 not 37

39. 35 not 38

Appendix 2: STROME-ID scores for the included studies

Author	STROME-ID score[a]
Aleksic, E	24
Alliex-Beguec, C	32
Allix-Beguec, C	25
Alonso-Rodriguez, N	18
Asgharzadeh, M	28
Bidovec-Stojkovic, U	31
De Beer, JL	30
Dymova, MA	19
Evans, J	[b]
Grujav, U	32
Guang-ming, DAI	19
Hamblion, E	[b]
Hang, NTHL	31
Jonsson, J	22
Lim, LKY	30
Mandal, S	32
Muwonge, A	25
Nikolayevsky, V	23
Oelemann, M	34
Ojo, OO	36
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[a]Individual studies score 1 for each element of checklist they had address

[b]Conference abstracts

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	n/a
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	appendix
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	4
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	5
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	5
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	5

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	5
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	5
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	15
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	15
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Figure 2
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	18
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	15
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	18
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	7
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	7-8
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	8
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	8

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