PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Effect of study design and setting on tuberculosis clustering estimates using Mycobacterial Interspersed Repetitive Units-Variable Number Tandem Repeats (MIRU-VNTR): A systematic review
AUTHORS	Mears, Jessica; Abubakar, Ibrahim; Cohen, Ted; McHugh, Timothy; Sonnenberg, Pamela

VERSION 1 - REVIEW

REVIEWER	Tomoshige Matsumoto, MD, PhD Osaka Anti-Tuberculosis Association Osaka Hospital, Neyagawa, Osaka, Japan
REVIEW RETURNED	05-Jul-2014

GENERAL COMMENTS	MIRU-VNTR analyses have some variations, 12-loci, 24-loci, old 12-loci and new 12-loci, which has different resolution HGDI even in the same area. So, it is not even to discuss these analyses as one MIRU-VNTR. Author should describe these analyses as each.

REVIEWER	Sathish Mundayoor Rajiv Gandhi Centre for Biotechnology Thycaud P.O. Thiruvananthapuram Kerala, INDIA
REVIEW RETURNED	15-Jul-2014

GENERAL COMMENTS	The major comment on the paper is that it has not been able to answer the question that it has set out to answer- on whether Study design or other variables affect the results of MIRU-VNTR typing and the conclusion drawn is that the number of studies are too few to really answer the question. The other conclusion that larger prevalence of positive TB cases and more number of patients or loci examined would lead to decreased clustering is both logical and expected. I cannot comment on the better quality standards, as I am not aware of the STROBE ID, but cannot imagine whole genome sequencing becoming a typing methodology in TB endemic regions

REVIEWER	Deborah J. del Junco, Associate Professor, Surgery & Epidemiology University of Texas Health Science Center - Houston USA
REVIEW RETURNED	04-Oct-2014

GENERAL COMMENTS	**General Comments for Authors:** The meta-analysis is clearly presented in this paper and adheres to many of the PRISMA guidelines. However, the consistency of the effect i.e., the "proportion of TB cases that are clustered" across the 14 studies assessed was not measured (e.g., by I^2 or a test for homogeneity) raising concern for whether a meta-regression was needed or appropriate. **Specific Comments for Authors:** 1. The synthesis of results (e.g., an overall summary proportion clustering and tests of the consistency across studies was not provided. If no statistically significant heterogeneity could be detected across the 14 studies (in the proportion of TB cases that were clustered), one could argue there was no justification for pursuing even a univariable meta-regression. 2. Provide a clear definition for the "proportion of clustering." For example, is it a. the number of recurring and genetically identical isolates (regardless of the number of repeat isolates contributing to each cluster)/total number of clustered and unique isolates or b. something else? 3. Is the proportion of clustering defined and reported consistently for each study in the meta-analysis? 4. Clarify whether or not a single individual within each study's source population could have multiple genetically distinct isolates, and if so, what was the size of each study's source population and what proportion had multiple isolates. 5. Write out Multidrug-Resistant for "MDR" on line 90, p. 4 6. Size of the study and maximum cluster size (lines 123-124, p. 5) would be expected to be correlated/non-independent covariates. 7. Does a low prevalence of culture positivity reflect a source population with a high prevalence of cases under prolonged treatment (i.e., prevalence cases)? Are culture positive cases more likely to be newly diagnosed (i.e., incidence cases) and not yet started on treatment? The inclusion of prevalence cases in some or all study populations could explain the nearly null association with TB incidence rates and the duration of each study. It would be helpful to clearly address these issues in the manuscript. 8. Clarify the definition of "prevalence of TB/HIV co-infection" in Table 1. Is it the prevalence of co-infection in each study's total source population, the prevalence among the subset of individuals with TB, or the prevalence among the subset of individuals with HIV? Is it defined consistently across all study reports included in the meta-analysis? 9. Line 96 on p. 4 describes data collection for and Table 2 reports a beta coefficient for the variable, "% clusters with 2 cases," but it would help if each study's results for the variable should be

	reported in Table 1. 10. The support in the findings for the authors' conclusion in the Discussion section, "We illustrate that the interpretation of studies using MIRU-VNTR to estimate clustering is subject to bias relating to study design," is unclear given the many limitations of the meta-analysis, especially the small sample size and questionable construct validity of the dependent and independent variables (see #11 below). 11. More discussion of the strengths and limitations of the "proportion of clustering" as it was operationally defined in this meta-analysis and the authors' rationale for each one of the covariates evaluated would have been helpful.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name Tomoshige Matsumoto, MD, PhD
Institution and Country Osaka Anti-Tuberculosis Association Osaka Hospital, Neyagawa, Osaka, Japan
Please state any competing interests or state 'None declared': Nothing to declare

Reviewer 1 comment: MIRU-VNTR analyses have some variations, 12-loci, 24-loci, old 12-loci and new 12-loci, which has different resolution HGDI even in the same area. So, it is not even to discuss these analyses as one MIRU-VNTR. Author should describe these analyses as each.

Author response: Thank you for this excellent point. To address this we conducted a sensitivity analysis excluding those studies that used the old 12 loci, to see if this changed the results. The results remained unchanged for all variables, apart from the significance of the relationship between the number of loci typed and the proportion of clustering. This is to be expected as the number of studies using 15 loci decreased from ten to six. An additional sensitivity analysis (not reported in the paper) which grouped studies using the 'old 12' loci, 'new 12' loci, and 24 loci found the effect. This sensitivity analysis is now described in the methods section, reported in the results section and discussed in the discussion.

Reviewer: 2
Reviewer Name Sathish Mundayoor
Institution and Country Rajiv Gandhi Centre for Biotechnology
Thycaud P.O.
Thiruvananthapuram
Kerala, INDIA
Please state any competing interests or state 'None declared': None Declared

Reviewer 2 comment: The major comment on the paper is that it has not been able to answer the question that it has set out to answer- on whether Study design or other variables affect the results of MIRU-VNTR typing and the conclusion drawn is that the number of studies are too few to really answer the question. The other conclusion that larger prevalence of positive TB cases and more number of patients or loci examined would lead to decreased clustering is both logical and expected.

I cannot comment on the better quality standards, as I am not aware of the STROBE ID, but cannot imagine whole genome sequencing becoming a typing methodology in TB endemic regions

Author response: Thank you for these comments. The certainty of our results has increased by identifying additional relevant studies that have been published since this paper first went to review. We now conclude 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. Whilst a lack of good quality reporting meant that we could not conduct a multivariable meta-regression to further explore the impact of study design and setting on the proportion clustered, we are confident that the findings we present are important for the interpretation of TB strain typing studies already conducted, and the design of future studies, therefore making a valuable contribution to the literature.

Reviewer: 3
Reviewer Name Deborah J. del Junco, Associate Professor, Surgery & Epidemiology
Institution and Country University of Texas Health Science Center - Houston
USA
Please state any competing interests or state 'None declared': None declared

General Comments for Authors:

Reviewer 3 comment: The meta-analysis is clearly presented in this paper and adheres to many of the PRISMA guidelines. However, the consistency of the effect i.e., the "proportion of TB cases that are clustered" across the 14 studies assessed was not measured (e.g., by I2 or a test for homogeneity) raising concern for whether a meta-regression was needed or appropriate.

Author response: Thank you for this important comment. We agree that the consistency of the effect should be measured and presented. The proportion of TB cases that are clustered across the 27 studies is now presented in a forest plot, by number of loci typed (figure 2 in the manuscript). There was strong evidence for heterogeneity between studies (chi2 test for heterogeneity was highly significant ($p<0.001$)), suggesting that a meta-regression would be an appropriate method of analysis. This is now described in the results section of the manuscript: "A forest plot shows the spread of clustering reported by number of loci and additional typing method (figure 2). The chi2 test of heterogeneity identified significant heterogeneity between the studies ($p<0.001$), suggesting that a meta-regression would be an appropriate analysis."

Specific Comments for Authors:

Reviewer 3 comment 1: The synthesis of results (e.g., an overall summary proportion clustering and tests of the consistency across studies was not provided. If no statistically significant heterogeneity could be detected across the 14 studies (in the proportion of TB cases that were clustered), one could argue there was no justification for pursuing even a univariable meta-regression.

Author response: Thank you for this excellent comment. As we have just explained in response to the comment above, we agree that the consistency of the effect should be measured and presented. The proportion of TB cases that are clustered across the 28 studies is now presented in a forest plot, by number of loci typed (figure 2 in the manuscript). There was strong evidence for heterogeneity between studies (chi2 test for heterogeneity was highly significant ($p<0.001$)), suggesting that a meta-regression would be an appropriate method of analysis. This is described in the results section of the manuscript: "A forest plot shows the spread of clustering reported by number of loci and additional typing method (figure 2). The chi2 test of heterogeneity identified significant heterogeneity between

the studies ($p<0.001$), suggesting that a meta-regression would be an appropriate analysis."

Reviewer 3 comment 2: Provide a clear definition for the "proportion of clustering." For example, is it
a. the number of recurring and genetically identical isolates (regardless of the number of repeat isolates contributing to each cluster)/total number of clustered and unique isolates or
b. something else?

Author response: The definition of the proportion of clustering is the number of clustered isolates/number of clustered+unique isolates. We have now defined this in the methods section. "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."
In the results section we now describe how studies defined clustering: "In all studies, clustered isolates were defined as having identical strain types based on 15 or 24 MIRU-VNTR strain types, with or without Spoligotyping. 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, four excluded isolates with missing loci, and the remaining 20 did not report how they dealt with missing loci."

Reviewer 3 comment 3: Is the proportion of clustering defined and reported consistently for each study in the meta-analysis?

Author response: Thank you for raising this issue. The measure reported across the studies was not consistent, with some studies reporting the transmission estimate using the n-1 method (number of clustered isolates-number of clusters/number of unique+clustered isolates) and some reporting the proportion of clustering (number of clustered isolates/number of unique+clustered isolates). To address this, we extracted data on the number of clustered isolates and number of unique isolates from each paper in order to calculate the proportion of clustering based on the following definition: number of clustered isolates/clustered+unique isolates. Thanks to your suggestion above, the definition of proportion clustered is now included in the methods: "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."

Reviewer 3 comment 4: Clarify whether or not a single individual within each study's source population could have multiple genetically distinct isolates, and if so, what was the size of each study's source population and what proportion had multiple isolates.

Author response: Thank you for this excellent suggestion. Only one study reported the inclusion of one repeat isolate from a patient (1/196=0.5% of sample). Ten studies reported that no repeat isolates from the same patient were included in the clustering analysis. The remaining 17 studies did not report whether repeat isolates were included or not. In the results section we now describe the different approaches to repeat isolates: "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."

Reviewer 3 comment 5: Write out Multidrug-Resistant for "MDR" on line 90, p. 4

Author response: Thank you, we have written out multidrug-resistant. The excerpt now reads: "e.g. random samples, studies using subsets of populations such as multidrug-resistant patients"

Reviewer 3 comment 6: Size of the study and maximum cluster size (lines 123-124, p. 5) would be expected to be correlated/non-independent covariates.

Author response: Thank you for this comment. Size of the study and maximum cluster size are correlated (correlation coefficient=0.57, p=0.01).

Reviewer 3 comment 7: Does a low prevalence of culture positivity reflect a source population with a high prevalence of cases under prolonged treatment (i.e., prevalence cases)? Are culture positive cases more likely to be newly diagnosed (i.e., incidence cases) and not yet started on treatment? The inclusion of prevalence cases in some or all study populations could explain the nearly null association with TB incidence rates and the duration of each study. It would be helpful to clearly address these issues in the manuscript.

Author response: Thank you for this excellent suggestion. Whether the sample included isolates from new, or new and chronic cases of TB is described in the results section of the manuscript. In addition, we report the number of studies that included or excluded isolates from patients that had previous TB. "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." This information has also been included in table 3 and is mentioned in the discussion, which reads: "This is likely to be because of a lack of good quality evidence: of the 28 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)." This was discussed briefly in the discussion: "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."
After updating the review, a further 13 studies have been included and a significant positive relationship between proportion of clustering and TB incidence has been identified across the studies (p=0.007).

Reviewer 3 comment 8: Clarify the definition of "prevalence of TB/HIV co-infection" in Table 1. Is it the prevalence of co-infection in each study's total source population, the prevalence among the subset of individuals with TB, or the prevalence among the subset of individuals with HIV? Is it defined consistently across all study reports included in the meta-analysis?

Author response: Thank you for pointing this out. Table 1 shows the prevalence of TB/HIV coinfection in the source population country. TB/HIV was not reported consistently across the studies, with most studies not reporting HIV prevalence at all (see table 2 in the manuscript). The data reported in table 1 are estimates of the TB/HIV coinfection in the study country, taken from Kruijshaar et al. (2011) and WHO TB country profiles. This has been clarified in the methods section of the manuscript: "As so few studies reported TB/HIV coinfection rates, these estimates for the study country were taken from an EU-wide survey and WHO country profiles." In addition, the legend of table 1 reads: "estimates from the literature of the prevalence of TB/HIV co-infection reported in the study country".

Reviewer 3 comment 9: Line 96 on p. 4 describes data collection for and Table 2 reports a beta coefficient for the variable, "% clusters with 2 cases," but it would help if each study's results for the variable should be reported in Table 1.

Author response: This is an excellent suggestion. We have now included the proportion of clusters with two cases in table 3 (previously table 2).

Reviewer 3 comment 10: The support in the findings for the authors' conclusion in the Discussion section, "We illustrate that the interpretation of studies proportion of clustering" as it was operationally

defined in this meta-analysis and the authors' rationale for each one of the covariates evaluated would have been helpful.

Author response: Thank you for this suggestion. The definition of 'proportion of clustering' used in this meta-analysis is the number of clustered isolates/number of unique+clustered isolates. This is described in the methods section, which reads: "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."
The rationale for including study design and study setting covariates is briefly summarised in the introduction, which reads "Published meta-analyses and modelling studies using IS6110 RFLP data show that the proportion of clustering observed can be affected by 1) study design (affecting the proportion of eligible cases that are included in the study); 2) features of the typing method (such as the ability to type isolates with low copy numbers); and 3) study setting (such as characteristics of the study population). For example, the proportion of clustering increases when the fraction of the total data sampled increases and when study duration increases." Each covariate is a known source of bias in the interpretation of RFLP clustering, or an epidemiological risk factor for clustering with MIRU-VNTR typing.

VERSION 2 – REVIEW

REVIEWER	Deborah J. del Junco, Associate Professor, Surgery & Epidemiology University of Texas Health Science Center Houston, TX USA
REVIEW RETURNED	21-Dec-2014

GENERAL COMMENTS	The authors have been very responsive to the Editor's and reviewers' suggestions. The near doubling of the sample size by updating the review and the other revisions have enhanced this well written manuscript. However, a few relatively minor concerns remain, especially about the regression modeling. They are listed below. **Specific comments for authors:** **1.** Lines 30-31, Abstract: Should "…explained 14%, 14%, 27% and 48%, respectively, and had…" be rewritten as "…explained 14%, 14%, 27% and 48%, respectively, <u>of between-study variation</u>, and had…"? **2.** Lines 156-158 and 161-162: There seems to be a contradiction between these two statements regarding whether or not the "proportion of the population with culture positive TB" was excluded or included in the regression analyses. **3.** Table 2 shows negative adjusted R squared values for several covariates. Please check whether these are correct. Do they suggest too-small sample sizes or very ill-fitting models? **4.** The Table 3 footnote "a" regarding the interpretation of the regression coefficient for "study duration" seems incorrect. The coefficient is negative, so an increase in duration would correspond to a decrease in the proportion of clustering (not an increase). **5.** Lines 175-176: The statement, "requiring consent to type patient isolates reduced the proportion of clustering" is incorrect

	and contradicts the statement in lines149-150 and the results reported in Table 3 (a positive coefficient of 0.38) suggesting a significant increase in the proportion of clustering. Please check this.

VERSION 2 – AUTHOR RESPONSE

Thank you for these excellent comments. Following your recommendations, we have made small amendments to the paper, improving the clarity of the paper and removing errors. Here we address each comment and show how we have incorporated the changes. We have attached two versions of the manuscript: one with track changes; the other 'clean', with all changes accepted.

1. Lines 30-31, Abstract: Should "…explained 14%, 14%, 27% and 48%, respectively, and had…" be rewritten as "…explained 14%, 14%, 27% and 48%, respectively, of between-study variation, and had…"?

Author response: Thank you for this suggestion. The sentence has been changed to read: "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"

2. Lines 156-158 and 161-162: There seems to be a contradiction between these two statements regarding whether or not the "proportion of the population with culture positive TB" was excluded or included in the regression analyses.

Author response: Thank you for pointing this out. The proportion of the population with culture positive TB was included in the metaregression analysis. The sentence on lines 157-9 has been corrected. It now reads: "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)."

3. Table 2 shows negative adjusted R squared values for several covariates. Please check whether these are correct. Do they suggest too-small sample sizes or very ill-fitting models?

Author response: These adjusted R2 values are correct. They are negative because the covariates explain less of the heterogeneity that would be expected by chance (negative adjusted R2 values are also possible in a logistic regression model). This is more likely to happen in a metaregression model because the numbers are quite small.

4. The Table 3 footnote "a" regarding the interpretation of the regression coefficient for "study duration" seems incorrect. The coefficient is negative, so an increase in duration would correspond to a decrease in the proportion of clustering (not an increase).

Author response: Thank you for identifying this error. This has now been changed to read: "E.g. for a one unit increase in maximum cluster size, the proportion of clustering increases by 0.2."

5. Lines 175-176: The statement, "requiring consent to type patient isolates reduced the proportion of clustering" is incorrect and contradicts the statement in lines149-150 and the results reported in Table 3 (a positive coefficient of 0.38) suggesting a significant increase in the proportion of clustering.

Please check this.

Author response: Thank you for identifying this error. Requiring consent to type patient isolates increased the proportion of clustering (coefficient=0.38, p=0.029), which was not expected. This has been amended and the sentence now reads: “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.”

Finally, appendix 1 and 2 have been referenced in the main text of the paper, as recommended by the Editorial Assistant.