PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	The global epidemiology of Neisseria gonorrhoeae in infertile populations: a protocol for a systematic review
AUTHORS	Chemaitelly, Hiam; Harfouche, Manale; Blondeel, Karel; Matsaseng, Thabo Christopher; Kiarie, James; Toskin, Igor; Abu-Raddad, Laith

VERSION 1 - REVIEW

REVIEWER	Pippa Scott University of Otago, Christchurch, New Zealand
REVIEW RETURNED	14-Sep-2018

GENERAL COMMENTS	This protocol is very well written and describes methods in a suitable amount of detail. There are only a few additions that I would suggest for this paper before publication in order to provide further clarity. Requested additions: 1)The authors have not addressed how they will interpret the results of this review in relation to causality. It would seem that in most eligible studies that presence/absence of gonorrhoea will be assessed after the onset/diagnosis of infertility. Please clarify how you will address this in their interpretation of findings and review limitations. A point should also be included on page 3, after the abstract, under limitations of the study. Please note that the review findings will still be valuable to the knowledge base. However, interpretation of these findings should acknowledge the timing of the assessment of gonorrhoea prevalence relative to the diagnosis of infertility. 2) Under eligibility criteria, page 6. a) Please add, for further clarity, that although literature reviews themselves are not eligible for inclusion in the review, that their bibliographies will still be searched (as stated at the end of the search strategy section) b) Please add in the text a brief explanation of the reasoning behind excluding studies with fewer than 10 participants, or assessing infections from tissues samples from the upper genital tract. It is possible for the reader to guess why, but it would be better to state tour reasons. 3) under study selection, page 8: Please clarify what is meant by "duplicate study findings will only be considered once using the

	more detailed report". I totally agree that the same data should not appear multiple times in a meta-analysis. However, do you mean that you will retain all reports for a single study during screening, and extract the most complete data for each outcome from whereever it is most completely reported? Or will you exclude some (less detailed) reports during screening? The former is likely better as sometimes multiple publications will sometimes each report one outcome/assessment method more clearly. However, it should be stated which is being used. 4) Risk of Bias assessment, page 8-9: regarding "Confidence in the body of evidence will be assessed by reporting the fraction....". It would be good to state that you will report, for each study, the assessed risk of bias in each domain. Reporting the fraction of studies with high risk of bias in 1,2,3 or 4 domains may lose important information, as studies with a risk of bias in different domains could have results biased in a different direction or to a different extent. Also, be aware that a study where use of antibiotics has been ascertained is not necessarily unbiased (e.g. prevelance may be low because most have received antibiotics, whether or not antibiotic usage was assessed or not). Risk of bias could also be investigated as a source of between trial heterogeneity. In summary, please emphasise that risk of bias will be carefully considered (in terms of possible magnitude and direction or bias) in the interpretation of the review findings. 4) Risk of Bias assessment, page 9: regarding "The precision of measure will be determined based on sample size....". Is this necessary, since this will be taken in to account by weighting in meta-analysis? Its also more statistical uncertainty rather than a bias in itself. 5) Data synthesis, page 9: I'm not sure what is meant by "Here the prevalence measure for the overall study will be replaced by stratified measures....". Could you clarify, possibly by adding an example? 6) Meta-regression, page 10. You might or might not have enough studies and statistical power to preform these meta-regression analyses. Please add that you will present data in forest plots stratified by each of there predictors as well (and possibly each of the risk of bias domains). Minor changes: 1) in abstract last sentence of methods and analysis: change to "and potential sources of between-study heterogeneity will be explored..."? 2) in "strengths and limitations, page 3, third point: clearer to say "opportunities to address" rather than "opportunities to attend to"? 3) In Data synthesis and analysis, page 9: change to "... (CIs) will be calculated using meta-analysis" rather than "meta-analyses"

REVIEWER	Abigail Norris Turner Ohio State University, USA
REVIEW RETURNED	22-Dec-2018

GENERAL COMMENTS	This protocol is generally clearly written and its publication will likely provoke some feedback from the scientific community. I am

	unclear how the systematic review as currently conceived will elucidate the link between gonorrhea and infertility. This is because the data the authors propose to synthesize will examine gonorrhea incidence among people diagnosed with infertility – meaning the gonorrhea infection follows rather than precedes the infertility diagnosis. They will also use prevalence studies which diagnose gonorrhea and infertility at the same time, which are probably also are not informative. This is because the current gonorrhea infection is unlikely to have been the cause of the infertility (which by WHO definition is only diagnosed after 12 months of unprotected sex without pregnancy). If the goal is truly just to describe the prevalence and incidence of gonorrhea among infertile men and women, then this review as written will do that, but I am not sure what public health value this has. In sum: the concern about gonorrhea and infertility is that untreated gonorrhea will cause infertility, whereas the design of this study will tell us about gonorrhea acquired after infertility. Abstract 1. Line 36: “Only one site of infection per assay type will be considered based on a predefined priority order.” – I assume the authors mean one anatomical site … but still the point of this sentence is not clear. For infertility, presumably urogenital infection is the highest priority, but this should be stated explicitly. Do you mean one biospecimen type? (e.g. urine, urethral, cervical, etc.)? This is not site of infection, which would typically be interpreted as genital, rectal or oral. It is not clear why, scientifically, the authors would limit the data to one site of infection per assay type. 2. The dates of the study should be included in the abstract 3. The abstract needs to be clearer that although many types of study designs and populations will be included, all included records will be from infertile populations. 4. Abstract Line 43: “Quality assessments will be conducted for all measures included in the systematic review” – using what method? 5. Abstract Line 45: “Meta-analyses will be implemented using DerSimonian-Laird random-effect models to estimate the mean prevalence of gonorrhea globally” – presumably the authors mean the ‘mean prevalence of gonorrhea globally AMONG INFERTILE POPULATIONS’, right? (Same comment for p. 6, line 13 in the Introduction) 6. Eligibility: “Any document reporting a measure of gonorrhea incidence and/or prevalence in infertile populations based on primary data will be eligible for inclusion” – as above, unclear how measuring gonorrhea INCIDENCE in infertile populations advances the research questions – presumably the working biological hypothesis is that gonorrhea precedes infertility, not the reverse (which is what would be captured in a study examining gonorrhea incidence in infertile people). This comment extends to the next section on study outcomes. 7. Risk of Bias assessment – the Cochrane tool is not well adapted to non-randomized trials – or at least, was not well adapted the last time I tried to use it for a systematic review that included diverse designs, like this one. The authors may need to consider an alternative tool. 8. The review will likely include many papers which test infertile people for current gonorrhea infection. But the gonorrhea which caused the tubal damage which led to the infertility may have occurred many years in the past. Gonorrhea can eventually clear without treatment, and the patient may never know s/he was

	infected. Similarly, a current gonorrhea infection, when detected in this setting, is unlikely to have been present long enough to have caused the infertility that brought the patient in for screening. These seem like major flaws with including this kind of study in the review.

VERSION 1 – AUTHOR RESPONSE

Reviewers' comments to the author:

Reviewer #1

Comments

This protocol is very well written and describes methods in a suitable amount of detail. There are only a few additions that I would suggest for this paper before publication in order to provide further clarity.

Comment: We thank the reviewer for assessing our work and for the constructive feedback on our manuscript that enriched the article and improved its readability. Please find below a point-by-point reply addressing each of the reviewer's comments.

Requested additions:

1) The authors have not addressed how they will interpret the results of this review in relation to causality. It would seem that in most eligible studies that presence/absence of gonorrhoea will be assessed after the onset/diagnosis of infertility. Please clarify how you will address this in their interpretation of findings and review limitations. A point should also be included on page 3, after the abstract, under limitations of the study. Please note that the review findings will still be valuable to the knowledge base. However, interpretation of these findings should acknowledge the timing of the assessment of gonorrhoea prevalence relative to the diagnosis of infertility.

Answer: We thank the reviewer for bringing up this excellent point. Strictly speaking, our study does not aim to investigate the causal link between gonorrhea infection and infertility. The study scope encompasses cross-sectional studies of gonorrhea prevalence among infertile populations, and thus prevents us from a direct investigation of causality. Prospective studies would provide a more direct investigation of causality, but are virtually impossible to conduct, for ethical reasons, as gonorrhea is a curable infection.

This being said, and though it will provide only an indirect and inconclusive evidence, the value of this work lies in its ability to demonstrate whether there is an association (but not necessarily causation)—i.e. much higher prevalence levels among infertile populations as compared to (our knowledge of) gonorrhea prevalence in general populations. If so, we would have suggestive evidence for a causal link, more so that current gonorrhea infection is often predictive of previous exposure to gonorrhea and vice versa.6-8 Most of these exposures are also asymptomatic and of unknown duration and persistence. We had to resort to this study design, simply because it has been very difficult to assess the causal link using other study designs and data sources.

In sum, strictly speaking, our study does not answer the question of causality, but provides supporting evidence for a potential link between this infection and development of infertility. We have now revised the manuscript to clarify this point along the lines discussed here (Introduction, Page 5, Paragraph 2 and Strengths and limitations of the study, Page 3).

2) Under eligibility criteria, page 6.

a) Please add, for further clarity, that although literature reviews themselves are not eligible for inclusion in the review, that their bibliographies will still be searched (as stated at the end of the search strategy section)

Answer: We thank the reviewer for the thoughtful suggestion. We have now revised this section to clarify that the bibliography lists of literature reviews will be hand searched for any additional articles that can be potentially relevant (Methods, Page 7, Paragraph 1).

b) Please add in the text a brief explanation of the reasoning behind excluding studies with fewer than 10 participants, or assessing infections from tissues samples from the upper genital tract. It is possible for the reader to guess why, but it would be better to state tour reasons.

Answer: We thank the reviewer for the valuable suggestions. We have now revised the manuscript to clarify these points by adding brief explanations (Methods, Page 7, Paragraph 1).

3) under study selection, page 8: Please clarify what is meant by "duplicate study findings will only be considered once using the more detailed report". I totally agree that the same data should not appear multiple times in a meta-analysis. However, do you mean that you will retain all reports for a single study during screening, and extract the most complete data for each outcome from whereever it is most completely reported? Or will you exclude some (less detailed) reports during screening? The former is likely better as sometimes multiple publications will sometimes each report one outcome/assessment method more clearly. However, it should be stated which is being used.

Answer: We thank the reviewer for the useful comment. As the reviewer has correctly guessed, we meant here that we will retain all reports of a study during screening and eventually extract the most complete data for each outcome from wherever it is most completely reported. We have now revised the manuscript to clarify this point along the lines discussed here (Methods, Page 8, Paragraph 2).

4) Risk of Bias assessment, page 8-9: regarding "Confidence in the body of evidence will be assessed by reporting the fraction....". It would be good to state that you will report, for each study, the assessed risk of bias in each domain. Reporting the fraction of studies with high risk of bias in 1,2,3 or 4 domains may lose important information, as studies with a risk of bias in different domains could have results biased in a different direction or to a different extent. Also, be aware that a study where use of antibiotics has been ascertained is not necessarily unbiased (e.g. prevelance may be low because most have received antibiotics, whether or not antibiotic usage was assessed or not). Risk of bias could also be investigated as a source of between trial heterogeneity. In summary, please emphasise that risk of bias will be carefully considered (in terms of possible magnitude and direction or bias) in the interpretation of the review findings.

Answer: We thank the reviewer for the useful and insightful comment. We apologize for the confusion, but we will study and report the risk of bias (ROB) for each domain for each study individually, in addition to the summary results for ROB across domains. We have now revised the manuscript to clarify this point (Methods, Page 10, Paragraph 1).

We also thank the reviewer for bringing a valid point about lower prevalence due to exposure to antimicrobials. Indeed, we plan also to assess all ROB domains as a source of heterogeneity in prevalence in forest plots, meta-analyses, and univariable and multivariable meta-regressions. We will also interpret the findings in light of the results of the ROB and precision assessments. We have now revised the manuscript to comment on this point along the lines discussed here (Methods, Page 10, Paragraphs 2-3, and Methods, Page 11, Paragraphs 1&3).

5) Risk of Bias assessment, page 9: regarding "The precision of measure will be determined based on sample size....". Is this necessary, since this will be taken in to account by weighting in meta-analysis? Its also more statistical uncertainty rather than a bias in itself.

Answer: We thank the reviewer for the useful comment. We agree with the reviewer that sample size will be taken into account during weighting in meta-analysis, however, we are interested in this measure of precision as a source of heterogeneity to investigate the existence of a small-study effect. We have now revised the manuscript to clarify this point (Methods, Page 10, Paragraphs 2-3, and Methods, Page 11, Paragraphs 1&3).

6) Data synthesis, page 9: I'm not sure what is meant by "Here the prevalence measure for the overall study will be replaced by stratified measures....". Could you clarify, possibly by adding an example?

Answer: We thank the reviewer for the useful comment. We meant here to say that, in meta-analysis, the overall gonorrhea prevalence, for instance in infertile women or in infertile men, will be replaced with stratified measures such as by infertility diagnosis for each population. We have now incorporated an example to clarify this point as per the reviewer's recommendation (Methods, Page 10, Paragraph 4).

7) Meta-regression, page 10. You might or might not have enough studies and statistical power to preform these meta-regression analyses. Please add that you will present data in forest plots stratified by each of there predictors as well (and possibly each of the risk of bias domains).

Answer: We thank the reviewer for the excellent suggestion. We have now revised the manuscript to specify that forest plots will be produced to visualise prevalence measures and their 95% confidence intervals (CIs) stratified by key study and population characteristics, as well as ROB and precision domains (Methods, Page 10, Paragraph 3).

Minor changes:

8) in abstract last sentence of methods and analysis: change to "and potential sources of between-study heterogeneity will be explored..."?

Answer: We thank the reviewer for the useful suggestion. We have now incorporated this change as per the reviewer's recommendation (Abstract, Page 2, Paragraph 2, and Methods, Page 11, Paragraph 3).

9) in "strengths and limitations, page 3, third point: clearer to say "opportunities to address" rather than "opportunities to attend to"?

Answer: We thank the reviewer for the useful suggestion. We have now incorporated this change as per the reviewer's recommendation (Strengths and limitations of the study, Page 3).

10) In Data synthesis and analysis, page 9: change to "... (CIs) will be calculated using meta-analysis" rather than "meta-analyses"

Answer: We thank the reviewer for the useful comment. We have now incorporated this change as per the reviewer's recommendation (Methods, Page 10, Paragraph 4).

Reviewer #2

Comments

This protocol is generally clearly written and its publication will likely provoke some feedback from the scientific community.

Comment: We thank the reviewer for assessing our work and for the constructive feedback on our manuscript that enriched the article and improved its readability. Please find below a point-by-point reply addressing each of the reviewer's comments.

I am unclear how the systematic review as currently conceived will elucidate the link between gonorrhea and infertility. This is because the data the authors propose to synthesize will examine gonorrhea incidence among people diagnosed with infertility – meaning the gonorrhea infection follows rather than precedes the infertility diagnosis. They will also use prevalence studies which diagnose gonorrhea and infertility at the same time, which are probably also are not informative. This is because the current gonorrhea infection is unlikely to have been the cause of the infertility (which by WHO definition is only diagnosed after 12 months of unprotected sex without pregnancy). If the goal is truly just to describe the prevalence and incidence of gonorrhea among infertile men and women, then this review as written will do that, but I am not sure what public health value this has. In sum: the concern about gonorrhea and infertility is that untreated gonorrhea will cause infertility, whereas the design of this study will tell us about gonorrhea acquired after infertility.

Answer: We thank the reviewer for bringing up this excellent point. Strictly speaking, our study does not aim to investigate the causal link between gonorrhea infection and infertility. The study scope encompasses cross-sectional studies of gonorrhea prevalence among infertile populations, and thus prevents us from a direct investigation of causality. Prospective studies would provide a more direct investigation of causality, but are virtually impossible to conduct, for ethical reasons, as gonorrhea is a curable infection.

This being said, and though it will provide only an indirect and inconclusive evidence, the value of this work lies in its ability to demonstrate whether there is an association (but not necessarily causation)—i.e. much higher prevalence levels among infertile populations as compared to (our knowledge of) gonorrhea prevalence in general populations. If so, we would have suggestive evidence for a causal link, more so that current gonorrhea infection is often predictive of previous exposure to gonorrhea and vice versa.6-8 Most of these exposures are also asymptomatic and of unknown duration and persistence. We had to resort to this study design, simply because it has been very difficult to assess the causal link using other study designs and data sources.

In sum, strictly speaking, our study does not answer the question of causality, but provides supporting evidence for a potential link between this infection and development of infertility. We have now revised the manuscript to clarify this point along the lines discussed here (Introduction, Page 5, Paragraph 2 and Strengths and limitations of the study, Page 3).

Abstract

1. Line 36: "Only one site of infection per assay type will be considered based on a predefined priority order." – I assume the authors mean one anatomical site … but still the point of this sentence is not clear. For infertility, presumably urogenital infection is the highest priority, but this should be stated explicitly. Do you mean one biospecimen type? (e.g. urine, urethral, cervical, etc.)? This is not site of infection, which would typically be interpreted as genital, rectal or oral. It is not clear why, scientifically, the authors would limit the data to one site of infection per assay type.

Answer: We thank the reviewer for the thoughtful comment. We apologize for the confusion. As correctly indicated by the reviewer, we were referring here to biospecimen type rather than anatomical

site. We have now amended this statement as per the reviewer's recommendation (Abstract, Page 2, Paragraph 2). We have also explicitly stated that our main interest is in studies reporting current gonorrhea urogenital infections (Methods, Page 7, Paragraphs 1-3).

2. The dates of the study should be included in the abstract.

Answer: We thank the reviewer for the useful comment. We have now incorporated the dates of the study in the abstract as per the reviewer's recommendation (Abstract, Page 2, Paragraph 2).

3. The abstract needs to be clearer that although many types of study designs and populations will be included, all included records will be from infertile populations.

Answer: We thank the reviewer for bringing up this point. We have now revised the abstract to stress that the study focuses only on infertile populations (throughout the Abstract).

4. Abstract Line 43: "Quality assessments will be conducted for all measures included in the systematic review" – using what method?

Answer: We thank the reviewer for the comment. We apologize for not being able to include this information in the abstract due to the restricted word count, however, we have now clarified this point in the Methods of this manuscript (Methods, Page 9, Paragraph 2).

5. Abstract Line 45: "Meta-analyses will be implemented using DerSimonian-Laird random-effect models to estimate the mean prevalence of gonorrhea globally" – presumably the authors mean the 'mean prevalence of gonorrhea globally AMONG INFERTILE POPULATIONS', right? (Same comment for p. 6, line 13 in the Introduction)

Answer: We thank the reviewer for the useful comment. Indeed—we have now revised the Abstract and the manuscript to clarify this issue (Abstract, Page 2, Paragraph 2, and Introduction, Page 5, Paragraph 1).

6. Eligibility: "Any document reporting a measure of gonorrhea incidence and/or prevalence in infertile populations based on primary data will be eligible for inclusion" – as above, unclear how measuring gonorrhea INCIDENCE in infertile populations advances the research questions – presumably the working biological hypothesis is that gonorrhea precedes infertility, not the reverse (which is what would be captured in a study examining gonorrhea incidence in infertile people). This comment extends to the next section on study outcomes.

Answer: We thank the reviewer for the valuable comment. We agree with the reviewer that gonorrhea incidence in infertile populations does not add meaningfully to this study. Therefore, we now have revised the methodology to drop inclusion of incidence (Methods, Page 7, Paragraphs 2-3).

7. Risk of Bias assessment – the Cochrane tool is not well adapted to non-randomized trials – or at least, was not well adapted the last time I tried to use it for a systematic review that included diverse designs, like this one. The authors may need to consider an alternative tool.

Answer: We thank the reviewer for the valuable comment. We agree with the reviewer that the Cochrane tool is more suited for randomized clinical trials. However, our risk of bias assessment does not strictly use the Cochrane tool, but is an adaptation that is informed by the Cochrane approach9 and by existing systematic reviews of prevalence studies5 10-12. We have now revised the manuscript to clarify this point along the lines discussed here (Methods, Page 9, Paragraph 2).

8. The review will likely include many papers which test infertile people for current gonorrhea infection. But the gonorrhea which caused the tubal damage which led to the infertility may have occurred many years in the past. Gonorrhea can eventually clear without treatment, and the patient may never know s/he was infected. Similarly, a current gonorrhea infection, when detected in this

setting, is unlikely to have been present long enough to have caused the infertility that brought the patient in for screening. These seem like major flaws with including this kind of study in the review.

Answer: We thank the reviewer for bringing up this excellent point. As indicated above, strictly speaking, our study does not aim to investigate the causal link between gonorrhea infection and infertility. The study scope encompasses cross-sectional studies of gonorrhea prevalence among infertile populations, and thus prevents us from a direct investigation of causality. Prospective studies would provide a more direct investigation of causality, but are virtually impossible to conduct, for ethical reasons, as gonorrhea is a curable infection.

This being said, and though it will provide only an indirect and inconclusive evidence, the value of this work lies in its ability to demonstrate whether there is an association (but not necessarily causation)—i.e. much higher prevalence levels among infertile populations as compared to (our knowledge of) gonorrhea prevalence in general populations. If so, we would have suggestive evidence for a causal link, more so that current gonorrhea infection is often predictive of previous exposure to gonorrhea and vice versa.6-8 Most of these exposures are also asymptomatic and of unknown duration and persistence. We had to resort to this study design, simply because it has been very difficult to assess the causal link using other study designs and data sources.

In sum, strictly speaking, our study does not answer the question of causality, but provides supporting evidence for a potential link between this infection and development of infertility. We have now revised the manuscript to clarify this point along the lines discussed here (Introduction, Page 5, Paragraph 2 and Strengths and limitations of the study, Page 3).

VERSION 2 – REVIEW

REVIEWER	Pippa Scott University of Otago, New Zealand
REVIEW RETURNED	13-Mar-2019

GENERAL COMMENTS	Thank you for thoughtfully addressing each point raised by the reviewers.

REVIEWER	Abigail Norris Turner Ohio State University, USA
REVIEW RETURNED	15-Mar-2019

GENERAL COMMENTS	The revised manuscript includes new text addressing the issues raised previously. I remain skeptical about the value of such a massive undertaking to examine an association which will provide (at best) quite indirect evidence of an association between gonorrhea and infertility. The argument made by the authors is that observing a positive association between current gonorrhea and current infertility would suggest gonorrhea as a cause of infertility (even though the current infection, temporally, cannot be the cause of the current infertility in these patients), because people with current gonorrhea are likely to have also been infected with gonorrhea in the past. I agree that ethical considerations prohibit a prospective evaluation of the association between gonorrhea and infertility, but it seems like retrospective cohort

	studies linking past gonorrhea to subsequent fertility would be possible. In any case, a massive systematic review with the current design & data seems like a huge amount of effort to arrive a conclusion which is so limited. However, the revision is now clearer about this limitation and so I wish them luck with the project. I found one new mistake (p. 25, line 34): The infections are asymptomatic, but the exposures are not asymptomatic.