PEER REVIEW HISTORY

This paper was submitted to the STI but declined for publication following peer review. The authors addressed the reviewers' comments and submitted the revised paper to BMJ Open where it was re-reviewed and accepted.

ARTICLE DETAILS

TITLE (PROVISIONAL)	A cross-sectional study of Mycoplasma genitalium infection and correlates in women undergoing population based screening or clinic based testing for Chlamydia infection in London
AUTHORS	Svenstrup, Helle; Dave, Sangeeta ; Carder, Caroline; Grant, Paul; Morris-Jones, Stephen; Kidd, Ian; Stephenson, Judith

VERSION 1 - REVIEW

REVIEWER	Simms, Ian Health Protection Agency, HIVandSTI
REVIEW RETURNED	24-Jun-2013

GENERAL COMMENTS	Many thanks for giving me the opportunity to look through the manuscript. I have made a number of suggestions that I hope you will find useful in your revision. 1 Title. This doesn't reflect the datasources that are presented in the paper as neither the GUM and NCSP data provide estimates of prevalence. 2 Key messages. Point 1 - I can't see an acknowledgement for the NCSP in the manuscript - it would be nice to see appropriate recognition for the PCT that supplied the information. Point 2 - the age range for the women should be stated. Point 4 - this needs to be re-thought as Mg and Ct have different therapeutic regimes. 3 Abstract - this should be revised in line with comments made on the Key Messages. The terms "prevelance" and "risk factors" (which implies causality) should not be used in the abstract and throughout. 4 Introduction - This appears to contain quite a few different strains of thought. I suggest that you concentrate on providing information that is directly relevant to supporting the aim of the study presented here and justifying why another clinical service based study of positivity is required. 5 Methods - a full description of the datasets, their characteristics, biases and the clincial services from which they were taken should be given. This is required as the NCSP data could come from a variety of low risk settings. The potential problem of duplication between the datasets should also be addressed. The authors also need to justify why they included children between the ages of 9 and 15. In view of the results from this group I would be inclined to drop the age group from the analysis. 6 Discussion - the Discussion needs to be restructured to clearly state the key findings, biases and how these relate to the literature. The authors could then speculate on the likely public health importance of Mg and the information gap that exists between current knowledge and an effective intervention strategies. Given

	what we know currently about Mg it presents a very different picture to that seen for chlamydial infection.

- The manuscript received two reviews at STI but the other reviewer had declined to make the review public.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Comments to the Author
Many thanks for giving me the opportunity to look through the manuscript.
I have made a number of suggestions that I hope you will find useful
in your revision.

1 Title. This doesn't reflect the datasources that are presented in
the paper as neither the GUM and NCSP data provide estimates of prevalence.

Response: We have changed the title to 'A cross-sectional study of Mycoplasma genitalium prevalence and correlates in women attending a national chlamydia screening programme or sexually transmitted infection clinics in London'.

2 Key messages. Point 1 - I can't see an acknowledgement for the NCSP
in the manuscript - it would be nice to see appropriate recognition for
the PCT that supplied the information. Point 2 - the age range for the
women should be stated. Point 4 - this needs to be re-thought as Mg
and Ct have different therapeutic regimes.

Response: We have added this under acknowledgements, page 20.

3 Abstract - this should be revised in line with comments made on the
Key Messages. The terms "prevelance" and "risk factors" (which implies
causality) should not be used in the abstract and throughout.

Response: We have replaced 'risk factor' with 'correlate'. We think 'prevalence' is a correct term to use for describing our results but have also used 'frequency' when appropriate.

4 Introduction - This appears to contain quite a few different strains
of thought. I suggest that you concentrate on providing information
that is directly relevant to supporting the aim of the study presented
here and justifying why yet another clinical service based study of
positivity is required.

Response: We have restructured the introduction in response to your advice.

5 Methods - a full description of the datasets, their characteristics,
biases and the clincial services from which they were taken should be
given. This is required as the NCSP data could come from a variety of
low risk settings. The potential problem of duplication between the
datasets should also be addressed. The authors also need to justify
why they included children between the ages of 9 and 15. In view of
the results from this group I would be inclined to drop the lowere age
group from the analysis.

Response: We have addressed the comment about datasets etc. in the methods section on page 7 and have restricted our analysis to those aged 15 years and above in response to comment 2 by reviewer 2 on age of sample.

6 Discussion - the Discussion needs to be restructured to clearly state the key findings, biases and how these relate to the literature. The authors could then speculate on the likely public health importance of Mg and the information gap that exists between current knowledge and an effective intervention strategies. Given what we know currently about Mg it presents a very different picture to that seen for chlamydial infection.

Response: we have restructured the discussion in response to comments by both reviewers 1 and 2.

VERSION 2 – REVIEW

REVIEWER	Dr Ian Simms Public Health England
REVIEW RETURNED	11-Nov-2013

GENERAL COMMENTS	I have previously reviewed the manuscript for Sexually Transmiitted Infections. The authors have revised the manuscript well but one problem remains. This is concerned with the use of the phrase 'prevalence ..... in the general population'. The NCSP is not a general population sample and therefore it is inaccurate to infer that the NCSP dataset is the same as a general population sample. I would avoid the use of prevalence as this is potentially misleading. The manuscript has been revised well but one problem remains. This is concerned with the use of the phrase 'prevalence ..... in the general population'. The NCSP is not a general population sample and therefore it is inaccurate to infer that the NCSP dataset is the same as a general population sample. I would avoid the use of prevalence as this is potentially misleading.

REVIEWER	Paddy Horner University of Bristol I undertake consultancy work for a number of commercial companies providing diangostic tests for bacterial STIs
REVIEW RETURNED	20-Nov-2013

GENERAL COMMENTS	This is an interesting paper which has been revised following my previous comments. I believe it is an informative substantial piece of work which merits publication. I have the following comments Introduction page 6 lines 3-8: I disagree with this statement. I am not aware that genotypic variations in wild type M. genitalium has an effect on the likelihood of antimicrobial resistance developing with azithromycin 1 grm. The rates for azithromycin quoted in the previous sentence are for efficacy in wild type M. genitalium. The recent publications by angarius et al Tagg et al and Twin (38). Indicate that resistance develops following 1 grm treatment and that

	in some populations macrolide resistance is endemic. If macrolide resistance is present a prolonged course of azithromycin will not be effective. This sentence needs to be revised. Anagrius C, Loré B, Jensen JS. Treatment of Mycoplasma genitalium. Observations from a Swedish STD Clinic. PLoS ONE 2013; 8(4): e61481. Tagg KA, Jeoffreys NJ, Couldwell DL, Donald JA, Gilbert GL. Fluoroquinolone and Macrolide Resistance-Associated Mutations in Mycoplasma genitalium. Journal of Clinical Microbiology 2013; 51(7): 2245-9. Page 17 line 22: typo NCSP Page 17 lines 40-51: The first sentence does not make sense. It looks to me that in the NCSP sample MG was significantly less likely to be detected than CT (analysis not provided in the results)whereas this was not the case in GUM patients with if anything MG being more common in the 15-19 yr olds although this not significant p=0.28. Page 18 lines 31-37. This sentence does not make sense. No statistics are presented on this in the results but I note that the 95% CI for prevalences overlap so assume that the difference was not significant. I find this result difficult to explain as the majority of studies in women find CT more common than MG . 716 women were tested - study duration is surely not relevant its the number tested which is important when considering random variation. CT screening is certainly a possibility which could explain this esp as our recent paper on CT serology suggests CT screening may be having some effect at the population level. But this as you state is speculation (Horner et al PLoS ONE. 2013;8(8):e72001) Page 19 lines 22-27: I would quote the Angarius 2013 paper as well. Do you not also mean that azithromycin 1grm has been associated with development of MG macrolide resistance in some studies predominantly symptomatic men.(see above) If 9% of women are indeed co-infected there is a risk that azithromycin 1 grm for treatment of CT may inadvertently by promoting macrolide antimcrobial resistance in MG. This requires further study before firm conclusions can be drawn but I believe is a valid cause for concern. Population based studies have previously shown co-infection in <5% you should discuss this on page 17 line 18-20 Walker J, Fairley CK, Bradshaw CS, et al. The difference in determinants of Chlamydia trachomatis and Mycoplasma genitalium in a sample of young Australian women. BMC Infectious Diseases 2011; 11: 35. Oakeshott P, Aghaizu A, Hay P, et al. Is Mycoplasma genitalium in women the "New Chlamydia?" A community-based prospective cohort study. Clinical Infectious Diseases 2010; 51(10): 1160-6. Manhart LE, Holmes KK, Hughes JP, Houston LS, Totten PA. Mycoplasma genitalium among young adults in the United States: an emerging sexually transmitted infection. American Journal of Public Health 2007; 97(6): 1118-25. Andersen B, Sokolowski I, Ostergaard L, Kjolseth Moller J, Olesen F, Jensen JS. Mycoplasma genitalium: prevalence and behavioural risk factors in the general population. Sexually Transmitted Infections 2007; 83(3): 237-41.

VERSION 2 – AUTHOR RESPONSE

Reviewer Name Dr Ian Simms
Institution and Country Public Health England Please state any competing interests or state ‘None declared’: None declared

I have previously reviewed the manuscript for Sexually Transmiitted Infections. The authors have revised the manuscript well but one problem remains. This is concerned with the use of the phrase 'prevalence ..... in the general population'. The NCSP is not a general population sample and therefore it is inaccurate to infer that the NCSP dataset is the same as a general population sample. I would avoid the use of prevalence as this is potentially misleading.

Author response: We have replaced the phrase prevalence with frequency, infection or estimate throughout the manuscript unless it is applied to a defined population.

Reviewer Name Paddy Horner
Institution and Country University of Bristol Please state any competing interests or state ‘None declared’: I undertake consultancy work for a number of commercial companies providing diangostic tests for bacterial STIs

This is an interesting paper which has been revised following my previous comments. I believe it is an informative substantial piece of work which merits publication.

I have the following comments

Introduction page 6 lines 3-8: I disagree with this statement. I am not aware that genotypic variations in wild type M. genitalium has an effect on the likelihood of antimicrobial resistance developing with azithromycin 1 grm. The rates for azithromycin quoted in the previous sentence are for efficacy in wild type M. genitalium. The recent publications by angarius et al Tagg et al and Twin (38). Indicate that resistance develops following 1 grm treatment and that in some populations macrolide resistance is endemic. If macrolide resistance is present a prolonged course of azithromycin will not be effective. This sentence needs to be revised.
Anagrius C, Loré B, Jensen JS. Treatment of Mycoplasma genitalium. Observations from a Swedish STD Clinic. PLoS ONE 2013; 8(4): e61481.
Tagg KA, Jeoffreys NJ, Couldwell DL, Donald JA, Gilbert GL. Fluoroquinolone and Macrolide Resistance-Associated Mutations in Mycoplasma genitalium. Journal of Clinical Microbiology 2013; 51(7): 2245-9.

Author response: We have removed this sentence and replaced it with ' Resistance has been shown to develop following 1g of azithromycin and macrolide resistance is endemic in some populations and added in the references.

Page 17 line 22: typo NCSP
Author response: corrected

Page 17 lines 40-51: The first sentence does not make sense. It looks to me that in the NCSP sample MG was significantly less likely to be detected than CT (analysis not provided in the results)whereas this was not the case in GUM patients with if anything MG being more common in the 15-19 yr olds although this not significant p=0.28.

Author response: We have removed this text.

Page 18 lines 31-37. This sentence does not make sense. No statistics are presented on this in the results but I note that the 95% CI for prevalences overlap so assume that the difference was not

significant. I find this result difficult to explain as the majority of studies in women find CT more common than MG . 716 women were tested - study duration is surely not relevant its the number tested which is important when considering random variation. CT screening is certainly a possibility which could explain this esp as our recent paper on CT serology suggests CT screening may be having some effect at the population level. But this as you state is speculation (Horner et al PLoS ONE. 2013;8(8):e72001)

Author response: We have removed this text and changed the preceding sentence to 'In lower risk non-STI clinic attendees such as college students infection has been shown to range from <1% to 5%.(5;31) which is in keeping with our estimate in the chlamydia screening population. '

Page 19 lines 22-27: I would quote the Angarius 2013 paper as well. Do you not also mean that azithromycin 1grm has been associated with development of MG macrolide resistance in some studies predominantly symptomatic men.(see above) If 9% of women are indeed co-infected there is a risk that azithromycin 1 grm for treatment of CT may inadvertently by promoting macrolide antimcrobial resistance in MG. This requires further study before firm conclusions can be drawn but I believe is a valid cause for concern.

Author response: We have added in the references and added 'This treatment dose has also been associated with the development of M.genitalium macrolide resistance in some studies of predominantly symptomatic men (refs). The risk of inadvertent M.genitalium antibiotic resistance in co-infected women who are treated for chlamydia with 1g of azithromycin is therefore potentially a cause for concern although further research is required to confirm this.'

Population based studies have previously shown co-infection in <5% you should discuss this on page 17 line 18-20 Walker J, Fairley CK, Bradshaw CS, et al. The difference in determinants of Chlamydia trachomatis and Mycoplasma genitalium in a sample of young Australian women. BMC Infectious Diseases 2011; 11: 35.
Oakeshott P, Aghaizu A, Hay P, et al. Is Mycoplasma genitalium in women the "New Chlamydia?" A community-based prospective cohort study. Clinical Infectious Diseases 2010; 51(10): 1160-6.
Manhart LE, Holmes KK, Hughes JP, Houston LS, Totten PA. Mycoplasma genitalium among young adults in the United States: an emerging sexually transmitted infection. American Journal of Public Health 2007; 97(6): 1118-25.
Andersen B, Sokolowski I, Ostergaard L, Kjolseth Moller J, Olesen F, Jensen JS. Mycoplasma genitalium: prevalence and behavioural risk factors in the general population. Sexually Transmitted Infections 2007; 83(3): 237-41.

Author response: We have added the references and changed the sentence to 'Only 0.5% of all the women had both C. trachomatis and M.genitalium infections. Among women who had C. trachomatis, 9% were co-infected with M.genitalium compared to <5% in population based studies (refs).'
Author response: Page 10, lines 43 to 48 - we have also rechecked our data and corrected NCSP upper age limit and mean ages of the women attending the two clinics.