PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	A retrospective analysis of infection and antimicrobial resistance patterns of Mycoplasma genitalium among pregnant women in the southwestern United States
AUTHORS	Stafford, Irene; Hummel, Kelsey; Dunn, James J.; Muldrew, Kenneth; Berra, Alexandra; Kravitz, Elizabeth; Gogia, Soumya; Martin, Irene; Munson, Erik

VERSION 1 – REVIEW

REVIEWER	Hamasuna, Ryoichi University of Occupational and Environmental Health, Kitakyushu, Japan, Urology
REVIEW RETURNED	15-Mar-2021

GENERAL COMMENTS	In this study, author showed 4 points, which included that the prevalence of MG among pregnant women in c USA was 5.7%, the risk factors detecting MG were young age and black race, the mutation rate related to macrolide-resistance was 30% (author described the rete was lower than other countries) and only one sample showed fluoroquinolone-resistance mutation on ParC. I understand the efforts of authors to collect specimens and to analyze the samples. However, I do not think that discussion or interpretation for findings in this study was sufficient. Firstly, authors selected the pregnant women as a target. As authors described in discussion, the important point is the relationship between MG infection and adverse reproductive outcome. However, authors described that the data of perinatal outcome was lack in the limitation of discussion. If so, why did authors examine pregnant women? If the perinatal outcome was lack, the population in this study was young women who can produce pregnancy and the data of young women regarding MG was not new. I think author should examine the perinatal outcome by reading hospital chart. This point is very important. The second, author examined antibiotic resistance by using detect genetic mutations as macrolide-resistance r fluoroquinolone-resistance. In addition, author described that the resistant rates were lower than those in other countries. However, authors did not describe antibiotic use in this study. If authors want to discuss the antibiotic resistance, the history of antibiotic use among target population was important. In addition, the recent paper showed that the prevalence of macrolide-resistant rate in pregnant women in South Africa was low (lawmen STI 2021.97(2):152-156). I hope authors discuss why were the macrolide-resistant rare in pregnant women of USA. It is easy that author also examine the hospital chart. The third, author detected that Mg infection was associated with co-

	infection with TV or GBS. It was interesting data. However, if author want to describe the relationship between MG ad other STI, author should describe the behaviors of target population. In addition, why was not C. trachomatis which was a most common pathogen in STI related to MG infection? Minor Abstract; M. genitalium itself is not STI. The infection of MG or MG is a pathogen of STI. The literatures regarding antibiotic resistance in references were little bit old. Recently, a lot of investigators showed new data of resistance rates in any countries. Please check new data and compare with author's data.

REVIEWER	Bissessor, Melanie University of Melbourne, Department of Public Health
REVIEW RETURNED	16-Mar-2021

GENERAL COMMENTS	This is an interesting article describing the clinical and laboratory characteristics of pregnant women infected with Mycoplasma genitalium. Abstract : This is well written and satisfactory Introduction: This is long and be reduced especially the first paragraph. Material and Methods: Description of the demographic variables is comprehensive to read and I am wondering if they could be referred to the table in the results section to shorten this content. The statistical analysis is sound Lines 125 and 126 seems odd and I am not sure if required? Results: Lines 141 to 143 have poorly constructed sentences. The results are well presented and the Tables represent the results well Discussion: The discussion does not fully discuss the results and compare and contrast the current literature completely. Instead it reads more like an introduction. For e.g. lines 172 to 182 are more relevant to an introduction rather than a discussion of a paper . Could the authors speculate what impact storage and degradation of specimens may have on the results obtained. Additionally, is there a multiplex PCR where gonorrhoea/chlamydia and MG may be tested simultaneously to reduce specimen loss ? Can the authors describe from the literature other treatment options for macrolie resistant MG in pregnant women e.g.pristinamycin

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Ryoichi Hamasuna, University of Occupational and Environmental Health, Kitakyushu, Japan

Comments to the Author:
In this study, author showed 4 points, which included that the prevalence of MG among pregnant women in c USA was 5.7%, the risk factors detecting MG were young age and black race, the mutation rate related to macrolide-resistance was 30% (author described the rete was lower than other countries) and only one sample showed fluoroquinolone-resistance mutation on ParC.

I understand the efforts of authors to collect specimens and to analyze the samples. However, I do not think that discussion or interpretation for findings in this study was sufficient.
Firstly, authors selected the pregnant women as a target. As authors described in discussion, the important point is the relationship between MG infection and adverse reproductive outcome. However, authors described that the data of perinatal outcome was lack in the limitation of discussion. If so, why did authors examine pregnant women? If the perinatal outcome was lack, the population in this study was young women who can produce pregnancy and the data of young women regarding MG was not new. I think author should examine the perinatal outcome by reading hospital chart. This point is very important.

Response 1

I agree that perinatal outcomes are incredibly important, however our aim was to determine characteristics of this infection among a pregnant cohort only, including prevalence rates, demographic factors, co-infection information and antimicrobial resistance patterns of *M. genitalium* positive swabs collected from pregnant women. Our first goal was to determine if *M. genitalium* infection in pregnant women share characteristics of *M. genitalium* infection in other females. Moreover, the number required to determine meaningful outcome data, i.e. preterm birth after adjusting for prior preterm birth using a conservative odds ratio of 1.3 per Lis et al. would require over 17,000 patients to determine a 30% difference in preterm birth, when using higher published prevalence rates among women of 15% and a macrolide resistance rate of 25%. The information provided in this manuscript can inform research scientists for future prospective studies including a large, randomized-controlled treatment trial to prevent preterm birth related to *M. genitalium* infection. This has been added to the discussion section of the manuscript.

Reviewer 1

The second, author examined antibiotic resistance by using detect genetic mutations as macrolide-resistance r fluoroquinolone-resistance. In addition, author described that the resistant rates were lower than those in other countries. However, authors did not describe antibiotic use in this study. If authors want to discuss the antibiotic resistance, the history of antibiotic use among target population was important. In addition, the recent paper showed that the prevalence of macrolide-resistant rate in pregnant women in South Africa was low (lawmen STI 2021.97(2):152-156). I hope authors discuss why were the macrolide-resistant rare in pregnant women of USA. It is easy that author also examine the hospital chart.

Response 2

I appreciate the reviewer's comments about prior antimicrobial use, macrolide and fluoroquinolone resistance pattern differences of *M. genitalium* isolated from pregnant women in the U.S. We did a chart review of all patients included and only recorded medications that were administered during the pregnancy and after the swab was collected. Prior antibiotic usage is not routinely asked at intake to care appointments given challenges with patient memory, health literacy and questionable relevance prior to STI testing. It is difficult to generalize why resistance patterns vary in different regions of the globe however this has been observed with many STI. For example, rates of syphilis in women in the US is 2.3 cases per 100,000 women (CDC STI surveillance data) vs. South Africa where 6% of women have syphilis (European Centre of Disease Prevention and Control)

Reviewer 1

The third, author detected that Mg infection was associated with co-infection with TV or GBS. It was interesting data. However, if author want to describe the relationship between MG ad other STI, author should describe the behaviors of target population. In addition, why was not C. trachomatis which was a most common pathogen in STI related to MG infection?

Response 3

We agree with the reviewer's comments about recording sexual history of the population. Unfortunately, as much as this is pertinent, sexual history data is plagued with inherent flaw given the issues surrounding patient disclosure and stigmatization of patient reporting of STI to American providers. Additionally, it is rarely reported in the literature reporting STI prevalence and antimicrobial resistance patterns.

Reviewer 1 Minor
Abstract; M. genitalium itself is not STI. The infection of MG or MG is a pathogen of STI.
The literatures regarding antibiotic resistance in references were little bit old. Recently, a lot of investigators showed new data of resistance rates in any countries. Please check new data and compare with author's data.

Response 4

This has been corrected. New references have been added

Reviewer: 2
Dr. Melanie Bissessor, University of Melbourne, Melbourne Sexual Health Centre
Comments to the Author:
This is an interesting article describing the clinical and laboratory characteristics of pregnant women infected with Mycoplasma genitalium.

Abstract : This is well written and satisfactory

Introduction: This is long and be reduced especially the first paragraph.

Response 1

This has been shortened.

Reviewer 2

Material and Methods: Description of the demographic variables is comprehensive to read and I am wondering if they could be referred to the table in the results section to shorten this content. The statistical analysis is sound

Response 2

This has been noted and the section has been abbreviated.

Reviewer 2

Lines 125 and 126 seems odd and I am not sure if required?

Response 3

The journal asked for this information, so it has been left in.

Reviewer 2

Results:
Lines 141 to 143 have poorly constructed sentences.

Response 4

This has been changed.

The results are well presented and the Tables represent the results well

Reviewer 2

Discussion:

The discussion does not fully discuss the results and compare and contrast the current literature completely. Instead it reads more like an introduction. For e.g. lines 172 to 182 are more relevant to an introduction rather than a discussion of a paper .

Response 5

This has been modified and lines 172 – 182 have been shortened significantly and more relevant discussion added – line 209-215

Reviewer 2

Could the authors speculate what impact storage and degradation of specimens may have on the results obtained. Additionally, is there a multiplex PCR where gonorrhoea/chlamydia and MG may be tested simultaneously to reduce specimen loss ?

Response 6

A sentence regarding the transfer of specimens across sites and possible degradation has been added 221-222. Hologic Panther platform testing options is described and referenced as a better option for further studies in 232-233 given the ability of the test to detect *M. genitalium*, gonorrhea and C. trachomatis using one swab. Unfortunately, to test for all organisms, we would have to change our study design to prospective and obtain informed consent to test for *M. genitalium* as it is not routinely tested for in the US.

Reviewer 2

Can the authors describe from the literature other treatment options for macrolide resistant MG in pregnant women e.g.pristinamycin

Response 7: This has been added to the discussion section. Starting line 199

Pristinamycin, an antimicrobial agent synthesized from macrolide and depsipeptide components, has demonstrated promising results as a second-line treatment option with a 75% cure rate of M. genitalium in preliminary studies. Although not significantly different from moxifloxacin in treatment efficacy among non-pregnant people, pristinamycin remains a potential option during pregnancy and in other situations where fluoroquinolones have failed or are contraindicated. Thank you very much

VERSION 2 – REVIEW

REVIEWER	Bissessor, Melanie University of Melbourne, Department of Public Health
REVIEW RETURNED	30-Apr-2021

GENERAL COMMENTS	References 10 and 11 have merged. This needs to be corrected. Thank you for making the changes to the suggestions I made