Author’s response to reviews

Title: Frequency and genotypes of Chlamydia trachomatis in patients attending the obstetrics and gynecology clinics in Jalisco, Mexico and correlation with sociodemographic, behavioral, and biological factors

Authors:

Néstor Casillas-Vega (nestor.casillas.vega@hotmail.com)
Rayo Morfín-Otero (rayomorfin@gmail.com)
Santos García (santos@microbiosymas.com)
Jorge Llaca-Díaz (jorgellaca@hotmail.com)
Eduardo Rodríguez-Noriega (idfcolima@yahoo.com)
Adrián Camacho-Ortiz (acamacho_md@yahoo.com)
Ma de la Merced Ayala-Castellanos (marimer66@yahoo.com.mx)
Héctor Maldonado-Garza (hectormaldonadog@yahoo.com)
Jesús Añer-Rodríguez (jesus.ancer@uanl.mx)
Guadalupe Gallegos-Ávila (guadalupe.gallegos@gmail.com)
Alberto Niderhauser-García (albertoniderhauser@gmail.com)
Elvira Garza-González (elvira_garza_gzz@yahoo.com)

Version: 1 Date: 16 Jun 2017

Author’s response to reviews:

Tovah Honor Aronin, Ph.D

Editor

BMC Women's Health

Thank you for your careful review of our manuscript entitled "Frequency and genotypes of Chlamydia trachomatis in patients attending the obstetrics and gynecology clinics in Jalisco,
Mexico and correlation with sociodemographic, behavioral, and biological factors." with the manuscript number BMWH-D-15-00073.

We have considered the reviewers’ comments and respond as follows:

Daman Saluja (Reviewer 1):

With increasing antimicrobial resistance, it is important that the host-pathogen interactions should be studied in detail. The first step is to identify the various strains and their association with specific symptoms.

Response: The most common symptoms as observed in these clinical samples, were PID (44.5%); dyspareunia: pain/burning (33.5%), and bleeding (4.2%); vaginal discharge: scarce (15.2%), moderate (48.6%), copious (27.9%), white (68%), yellow (29.3%), green (6.8%), and brown (2.8%); previous infection: urinary (28.4%), and vaginal (63.4%) (See Table 1). In this manuscript, significant associations of C. trachomatis infection with symptoms were reported in line 209, which were PID (n=36 (37.5%), p=0.04) and green vaginal discharge (n=45 (11.5%), p=0.04). Association of C. trachomatis genotypes with symptoms, [genotype F, L2 is associated with PID (p = 0.02), and genotype E was associated with green vaginal discharge (p = 001)], is mentioned in lines 21-218.

Work described is good and suggests that E and F serovars are most frequent in the population tested. This is in contrast to some of the other reports.

Response: The prevalence of C. trachomatis genotypes has been determined for several countries, but little such data are available for Latin America. In our study, the most frequent genotype was E (39.6%), followed by F (29.2%) and D (15.6%). In a previous study of C. trachomatis in Mexico, genotype F was most frequent (54.2%), followed by genotypes E, G, K, and L2 (8.7% each); genotypes D, F, and I were detected at a frequency of 4.2% each. That study, however, involved the examination of only 152 samples from infertile women (with only 24 specimens positive for C trachomatis), and limited clinical data were reported that contrasted with our results, this may be due to the small sample analyzed by the previous study. Our results are similar to those reported from other Latin American regions and other parts of the world. In a
Brazilian study that included 141 women, the most frequent genotype detected was E (39.7%), followed by F (17.7%) and D (17%) [21]. In a Costa Rican study, including 806 C. trachomatis–positive samples, genotype E were also most frequent (31%), followed by F and D (21% each). Genotype E was most frequent in a study including 81 women conducted in Argentina. Similar frequencies have been reported in other parts of the world, including the Netherlands (E, 41.5%; F, 21.8%; D, 11.9%), China (E, 37.2%; F, 31.3%), and Alabama, United States (E, 29%; F and D, 19% each).

In our population, genotype L2 was identified in three patients with no clinical data of LGV. This situation has been described previously in Mexico. LGV is a sexually transmitted disease caused by C. trachomatis serotypes L1, L2, and L3. It probably affects both sexes equally, although it has been reported more frequently in men, in whom the early manifestations of the disease are more evident. Men often display the acute form of LGV, whereas it is often detected in women when late-stage complications develop. Most cases in Europe and North America have been identified in men who have sex with men. A small number of genotype L cases have been reported in heterosexuals in the United Kingdom and Europe, including seven women in the UK since 2004, but these cases appear to be linked to male bisexual partners or sexual contact with those returning from endemic areas. This information is included in the discussion section on lines 239-264.

As regards to associations of C. trachomatis genotypes with risk factors in the study population, I feel about 100 samples is too low to associate the said serotype with a specific risk factor and symptom. This is rather a preliminary report in that direction and needs to done on a much larger cohort. Hence it would be better if this data is expanded on higher number of chlamydia positive samples.

Response: We agree with the reviewer that more positive samples would be desirable to present this association; however, due to the initial project approach and the frequency of this pathogen, it will be difficult to get these samples at the present time. Therefore, we have modified the manuscript and have stated that this information is preliminary due to the limitation of the number of samples, (line 301-302).
Silvia Bianchi (Reviewer 2):

This reviewer thinks that deep attention could be done on "material and methods" section.

Response: The section on "material and methods" was revised and better described, added the following:

- Line 108: This was a cross-sectional study

- Line 115: Tubes were stored at -20°C until analyzed.

- Lines 118-120: The concentration and purity of the extracted DNA were evaluated through a spectrophotometer (GENESYSTM 20, Thermo Fisher Scientific, Germany).

- Lines 137-138: Each run was accompanied by positive and negative control sample.

- Lines 139-143: The amplification products were visualized using electrophoresis on a 2.5% agarose gel stained with 1 µg/ml ethidium bromide, and compared with a standard (DNA Molecular Weight, Marker 100, Sigma Aldrich, St. Louis, MO, USA), and developed with a UV transilluminator (UVP BioImaging Systems Photodocumentator, Upland, CA).

- Line 151: Each run was accompanied by positive and negative control sample.

- Lines 153-157: The amplification products were visualized using electrophoresis on a 1.5% agarose gel stained with 1 µg/mL ethidium bromide, and compared with a standard (DNA Molecular Weight, Marker 1000, Sigma Aldrich, St. Louis, MO, USA), and developed with a UV transilluminator (UVP BioImaging Systems Photodocumentator, Upland, CA).
- Lines 161-164: using electrophoresis on a 1.5% agarose gel stained with 1 µg/mL ethidium bromide, and compared with a standard (DNA Molecular Weight, Marker 1000, Sigma Aldrich, St. Louis, MO, USA), and developed with a UV transilluminator (UVP BioImaging Systems Photodocumentator, Upland, CA).

You have to better describe the experiments that you have done and described. For example,

Lines 125-134: how long is the fragment amplified?

Response: The fragment amplified size was added in the line 133.

Lines 141-142: are you sure of the amplification condition here reported?

Response: Small modifications were made to the conditions previously described by Lan and collaborators for the amplification of the OmpA gene: for the initial denaturation the time increased (6 min to 7 min) and decreased the temperature (95 °C to 94 °C); the number of cycles decreased (49 to 40), and increased the time for denaturation (1 min to 3 min). This information is included in the material and methods section on line 144.

This reviewer thinks it is necessary that the Authors explain why have decided to use three different methods for the detection of infection. What is the reference method? The gold standard? The three methods are concordant or discordant? It is possible to analyze the concordance / discordance of the results in relation to the characteristics of the study population? It necessary to comment about these differences.

Response: The DFA method was used in the first instance for screening, since it is practical and cheap, is largely preferred in routine laboratory practice. Subsequently two PCRs were performed, one for the identification of C. trachomatis by amplification of the PLDESP gene; and after this, a semi-nested PCR was performed to detect the OmpA gene and to perform genotyping of C. trachomatis.
The false positives detected by DFA, could be originated because the kit used the anti-LPS monoclonal antibodies that can cross-react with non-chlamydial bacterial species. The semi-nested PCR, which the literature mentions has a sensitivity and specificity of 100%.

Final results were regarded as true positives if the semi-nested PCR was positive (gold standard). The overall agreement of DFA results with semi-nested PCR the validation parameters were as follows: sensitivity 96%, specificity 97.3%, negative predictive value 99.3%, positive predictive value 86.5%, and accuracy 97.3%. Comparing the PCR results of the PLDESP gene with semi-nested PCR the validation parameters were as follows: sensitivity 98.6%, specificity 100%, negative predictive value 99.6%, positive predictive value 100%, and accuracy 99.7% (This info was added to the manuscript on the lines 197-203).

We thank the reviewers for their comments in this review and hope that the responses and modifications help clarify the manuscript. We believe that the manuscript has been strengthened as a result of this process.

Thanks in advance for considering this work.

Sincerely,

Elvira Garza-González

Avenida Madero s/n Colonia Mitras Centro

Monterrey, Nuevo Leon, 64460, Mexico

Phone: +52 (81) 83 29 41 66

E-mail: elvira_garza_gzz@yahoo.com