Reviewer's report

Title: Chlamydia trachomatis serovar distribution in clinical urogenital specimens from Tunisian patients: high prevalence of C. trachomatis serovar E and mixed infections

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Reviewer: Sylvia Bruisten

Reviewer's report:

This manuscript ‘Chlamydia trachomatis serovar distribution in clinical urogenital specimens from Tunisian patients: high prevalence of C. trachomatis serovar E and mixed infections’ shows interesting work that provides for the first time typing data on Chlamydia for a North African country.

The main message is that mainly Chlamydia trachomatis serovar E types were found, irrespective of gender, calendar time, age or co-infections. The methods used, reverse hybridization, seem to yield very many mixed infections (21%), even with ocular types A and B. That has seldomly been described before and in combination with the fact that these A and B types were always detected in conjunction with serovar E, it seems probable that some cross hybridization plays a role, although the authors state that their method did not show cross reaction. Alternatively these mixed infections were missed in other studies.

In general it is advised to use ‘genovar’ in stead of ‘serovar, throughout the entire manuscript, since typing is not based on antibodies (serology: serovar) on cells but on analysis of the ompA gene (genomic data: genovar).

The inclusion of samples is not clear. It seems highly improbable that in almost twelve years time only 172 samples were tested in Sfax. It would be highly interesting what the (estimated) prevalence of C. trachomatis infection is among Tunisian patients in Sfax. In that way it could be seen how representative the sample set is that was tested and typed in this study.

Also not clearly described is what kind of samples were used: direct swab eluates or urines and how many of which. Were all samples collected in 2SP medium from the Roche kit?

Were these direct samples also used for culturing and subsequent DNA analysis, PCR amplification and reverse hybridization? This last option would explain the high positivity rate for typing (137 of 138 tested samples). However culture was not mentioned at all in the paper.

Table 3 is not clear, see also below.

The conclusion that women ‘significantly more often have single infection’ as stated in the abstract and in Results does not seem to be the best conclusion. More samples from men had been included (60%) than from females (40%).
Thus it is better to conclude that ‘men significantly more often have mixed infections’, as is also mentioned in the Discussion. So, within those with mixed infection there were 23 men (79%) and 6 women (21). For single infection this gender difference was less pronounced. See also comments below.

Specific comments on BMC-ID

Abstract:
Methods: please add how many samples were tested in total and what is the prevalence of C. trachomatis among your patients.
Line 47: add ‘a’ after ‘reported’

Introduction
The Introduction is concise and to the point.
Line 78 Change ‘know’ for ‘described’.

Materials and methods
Clinical C. trachomatis samples
Line 86: change ‘and’ to ‘and/or’
Please add information on the Cobas testing: how many µl of the 2-SP medium was needed for this step? In line 99 is stated that 200 µl was needed for the Qiagen column DNA extraction, so it might be that after the Cobas testing not enough medium was left to perform other DNA extractions?
Were these samples also cultured and subsequently used for DNA extraction? That is very important for the results, since many mixed infections were identified.

PCR amplification of the VS1-VS2 segment
Line 110: please specify the NLO and CT4 primers: what is their sequence? Were they published previously? In that case give the appropriate reference.

Reverse hybridization method
Line 128: Change sentence in: Washing ‘steps’ were performed between each ‘of the incubation’ steps.

Results.
Line 135: As mentioned in general comments, it seems very improbable that only 172 samples could be tested in a period of almost 12 years in a University clinic such as this one in Sfax. Please indicate how many patients were seen (each year) and how many were tested, and of those how many tested positive for C. trachomatis. This will provide the prevalence of C. trachomatis within this group of Tunisian patients.
Line 136: why were only 138 available for testing from the 172 positives? Were
the samples discarded? Was the 2-SP medium possibly used up?
Line 137: Why were there missing data on age?
Were the other parameters in table 3 complete (co-infection (?), infertility, NG infection?) for all 137 participants?
Line 138: please provide the median age and the confidence interval in stead of the mean age and the range.
Line 139: Change into: Thirty two individuals (23.9%) ‘were younger’ than 25 years.
Lines 139-141: Adding up 39+97+3 amounts to 139 patients and not to 138 or 137. Please review what are the correct numbers.
In addition, how many of these with current infection or infertility complaints were males and how many were females?
Line 146: skip ‘then’
Line 147: Skip ‘s’ in Serovars distribution, also in later sentences.
Line 148: A heterogeneous distribution ….. was observed
Line 151: Skip ‘in’
Line 152: add ‘together’ after infections
Line 153: replace ‘to be’ in stead of ‘for’ serovars E and F,
Line 157: The group distribution is shown in table 2, not in table 3
Line 160 and 161: Rewrite sentence for clarity. For example: All single and mixed genotypes were detected in the total study period of 12 years except for genovar D, A and B.
Line 166 to 168: This line gives too many results in one sentence. It would be more appropriate to state that significantly more men had mixed infections (p=0.02).
Table 3: The age data were available for only 115 persons. This should also be mentioned in the table legend.
Furthermore, it is not clear if more data were missing for other parameters such as infertility (see above).
What is meant by ‘infection’? Presence in 39, so 137-39=98 had no infection?
The B-group testing versus other seems the same as the E single test versus other, since the B-group mainly consists of genovar E (LGV was not present and almost no B)!
Still, there is a significant difference for gender but not for E-single versus other. I suggest to leave out the B-group test, it seems artificial.

Discussion
Line 177: As said above it is strange that only 172 samples were collected and tested in 12 years time: please explain.

Line 186: add after prevalent: , being

Line 193: add: not so in our case.

Line 195: areas

Line 201: change ‘sustaining’ in ‘persistence’ (if that is what was meant)

Lines 215-218: First it is stated that in the literature no more than 15% mixed infections is reported. Then ref 29 is mentioned where >50% mixed infections were found? Please clarify these statements. Indeed 21% is VERY high. So please review the original data that no spurious bands were included.

Line 219: the sentence that starts with ‘In our study’ is not clear. What is meant? Maybe: There were no patients of whom multiple samples were obtained at one visit to the clinic?

Line 221: different samples of anatomical sites (?).

Line 229: Change ‘Besides’ into ‘Also’ these serovars were involved in urogenital infections before. The refs 31 to 34 are all but one from more than 10 years ago. It remains intriguing that ocular strain are found in uro/genital samples!

Line 237: with ‘an’ other serovar

Lines 242-243: It is not clear from the Results how many infertile patients were found with what type of serovar: this should be added in the Results.

Possible this can be included in a Table. For example, in stead of the present Table 2 a new Table could be added that contains serovar distributions according to patient characteristics (gender, age, fertility, co-infections, etc).

Line 247: add ‘Table 3’ after serovar E.

Conclusion

Line 257: change into: high, much higher than in other reports in the world. We have also detected a high frequency..

Figure legend:
The genovar distribution is shown according to year of collection.

Table 1: Replace ‘serovars’ with ‘genovar’

Legend: Statistical significance is marked in bold.

Table 2: This Table can be omitted.

In stead a Table with genovar types according to patient characteristics should be included. Please be sure then to mention missing data.

Table 3: The last column concerning the B-group versus other can be omitted.
Missing data per category (age, infection, infertility, NG infection) should be mentioned in the Table legend. Furthermore: what is meant by ‘infection’?

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.