Author's response to reviews

Title: Genotypic determination of resistance and heteroresistance to clarithromycin in Helicobacter pylori isolates from antrum and corpus of Colombian symptomatic patients

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Version: 1 Date: 10 Dec 2018

Author's response to reviews:

Bogotá, December 10th 2018

Mrs.
Cecilia Devoto, PhD.
Editor

BMC Infectious Diseases

https://bmcinfectdis.biomedcentral.com/

Ref: Manuscript Submission: INFD-D-18-01256, entitled "Genotypic determination of resistance and heteroresistance to clarithromycin in Helicobacter pylori isolates from antrum and corpus of Colombian symptomatic patients".

I am submitting a revised version of the above manuscript. The following lists point by point the responses to the reviewers’ comments:

Technical Comments:
Comment 1. Title page. Please include the email addresses for all authors on the title page. The corresponding author should still be indicated. Please also ensure these email addresses match the email addresses provided in the editorial manager system.

Response: The email addresses for all authors was included on the title page (lines 4 to 7). Emails addresses are the same provided in the editorial manager system.

Reviewer reports:

First Reviewer. Avi Peretz

Comment 1. This is a very important issue. The antibiotic resistance rate of the bacterium is on the rise and therefore such work is important for determining the treatment of bacterial infection. The work is well written, clearly formulated, and the results are clearly presented. The methods are current, and the sample size is reasonable.

Response: Does not apply.

Second Reviewer. Marja Liisa Hänninen

Comment 1. The authors studied genetic CLA resistance among Colombian symptomatic patients with described demographic background. Both corpus and antrum isolates were studied from 126 paired H. pylori isolates. In addition, the isolates were studied using RAPD for genetic similarities of corpus and antrum isolates. The patient data is relevant and contains data on patients. This the strength of the manus. Genetic resistance to CLA was appr. 38%. This high level of genetic CLA resistance has been found in several other studies as well (e.g. Francesco et al. JAC 2010).

Usually phenotypic resistance is much lower than genetic resistance. The authors analysed not phenotypic resistance (MIC). This is a clear weakness of the study. Several previous studies have shown that among those patients having phenotypic resistance to CLA a low eradication rate with typical triple therapy is found (H. pylori is resistant). In opposite, the eradication rate among patients having only genetic mutations in 23rRNA gene but susceptible in phenotypic tests the eradication rate is high (H. pylori is susceptible).

These evidences mean that the conclusions of the authors on consequences of their results on therapy are too strong and are not based on the results presented. I suggest the authors include MIC data or the isolates in the manuscript. This is important and needed for interpretations of the results.

Response: Genotypic resistance that does not emerge phenotypically is mainly due to the detection of a heteroresistant status by the sequence method. Trespalacios et al, 2013
(https://link.springer.com/article/10.1007%2Fs12275-013-2465-6) showed a high degree of association between clarithromycin resistance assessed by agar dilution and point mutations in the peptidyltransferase of 23S rRNA gene in H. pylori Colombian strains. It was also identified that mutation A2142G showed higher levels of MICs (≥16 μg/ml) that the A2143G mutants (1 to 8 μg/ml), as has been previous reported. These findings were a confirmation of the results obtained by Alvarez et al., 2009 whom did a comparison between E-test and PCR (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2737890/). Therefore, considering the economic and time constraints, and additionally the difficulties to find a laboratory to do E-test (there were no laboratories in Bogotá (Colombia) that realize H. pylori culture or MICs in a conventional way), so we decided to consider it as a study limitation.

Francesco et al., JAC 2010 described lower eradication rates in phenotypic resistant strains than genotypic resistant but phenotypic susceptible strains, which is mainly due to the detection of the heteroresistance status by the molecular tests. However, Liou et al. 2011 (https://aac.asm.org/content/55/3/1123/article-info) found disagreeing results. Genotypic resistance (23S rRNA mutations) appeared to correlate better with therapeutic outcomes after clarithromycin-based triple therapy than the MICs from the agar dilution test. In a local context (again in Colombia), Trespalacios et al., in a chapter of his doctoral thesis (https://repository.javeriana.edu.co/bitstream/handle/10554/3935/tesis721.pdf?sequence=1&isAll owed=y page 138-173), report a percentage of eradication with the triple standard therapy of 21% in genotypic resistant bacteria versus 27,2% in phenotypic resistant isolates, with a therapy failure of 29% of which 65% were explained by the A2143G y A2142G mutations.

In our study, we found a low heteroresistance rate (7.9%) and additionally, most resistant cases harbor the A2143G mutation (14/19, the 22% of the sample), which has been directly related with a significative decrease in eradication therapy success. That’s why we suggest, following the international and national guides, which take a 15% of clarithromycin resistance as the upper limit to the prescription of triple therapy that include this antibiotic that it would be important to consider the application of quadruple therapy. However, as suggested, we modified manuscript at the lines 185-192, for a better explanation of the situation to the lectors.

Editorial comments

Comment 1. The language needs a total revision to improve English and make text better understandable

Response: Following the recommendation the manuscript was submitted to a language revision by Emily Harmon. Ms. Harmon has a MA in Teaching English as a Second Language. She currently works at Washington State University as an ESL instructor for international students matriculating into the university at both the undergraduate and graduate levels. She has extensive experience teaching English composition as well as assisting graduate students with research papers and dissertations. She read and reviewed this manuscript on December 5, 2018 and made multiple grammar revisions via Track Changes in Word as well as made suggestions via the comment feature to help clarify intended meaning through linguistic changes.
Comment 2. The gene you studied was 16 rRNA gene?.

Response: The gene we studied was 23S rRNA gene, this target allows the detection of H. pylori infection, but also provide information about antimicrobial susceptibility because it contains the peptidyl-transferase encoding region. Due to the double functionality, this gene is studied in almost all clarithromycin resistance researches.

Comment 3. Discussion needs a total revision. Too long and plenty of on discussion on topics were not relevant regarding the aims and results.

Response: As suggested by the reviewer, the discussion was revised, and length was reduced to highlight the relevant information.

We hope that these responses are satisfactory and look forward to hearing from you.

Yours sincerely,

Maria del Pilar Delgado

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