Format for ANSWERING REVIEWERS

December 14, 2016

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 31008-Revised manuscript.doc).

Title: Effect of treatment failure on the CagA EPIYA motif in *Helicobacter pylori* isolates

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 31008

The manuscript has been improved according to the suggestions of reviewers:

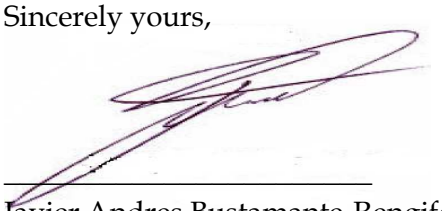
1. Format has been updated
2. The abstract was subtly modified for better compression by the reader.
3. The total volume of the manuscript was reduced on 30%, the sections mainly intervened were the introduction, methodology and discussion.
4. The introduction was modified to improve their understanding, and the aim of the study was emphasized.
5. The section of methods was modified reducing its extension
6. In "Material and methods", unnecessary parts were eliminated, reducing their extension.
7. In the methodology, it is clearly explained that the initial sample size was 206 participants from Túquerres, Colombia. Although not specified in the manuscript, these calculations were based on previous data suggesting an average prevalence of *cagA* and *vacA* genotypes of 89.3% and 79.0% in Túquerres (*High risk of gastric cancer*) and Tumaco (*Low risk of gastric cancer*) respectively, using a χ 2 test for two groups corrected for continuity, with a level of significance of 0.05%, 10% of losses and a power of 80%, resulting in a size sample of 206 participants in each population to detect statistically significant differences in the analyzes.
8. Additionally, at the beginning of the results, it is explained that of the 206 patients initially recruited, 176 and 149 participants were *H.pylori*-positive by histology and culture, respectively. Subsequently, 176 participants were treated. Six weeks after, it was possible to contact 174 participants to conduct the [^{13}C]-Urea breath test, nine of these participants were excluded. With the 165 participants in which was possible to conduct post-treatment control, it was found that 11 cases were ambiguous and 31 participants were [^{13}C]-UBT-positive. Of the participants with treatment failure, only 25 accepted to undergo a second endoscopy. Subsequently, 16 and 10 participants were *H.pylori*-positive by histology and culture, respectively. Although, we compare the isolates obtained before and after the therapeutic failure in only 10 participants, the results found in this study are of

great value because of the difficulties to conduct follow-up studies in vulnerable populations. Based on the study design, it is clarified that the number of patients with treatment failure can not be increased, as suggested a reviewer

9. In the results, specifically the section "*Antibiotic susceptibility and treatment failure*", it is mentioned that in all 149 isolates obtained before treatment, antibiotic susceptibility tests were performed. The results showed low rates of resistance to clarithromycin (2.7%) and amoxicillin (4%). In addition, it was observed that the isolates obtained from the 10 patients with treatment failure, only one of them (SV415) had previously shown isolates resistant to clarithromycin (MIC > 4.0 mg/L). This shows that although primary antibiotic resistance is an important characteristic *in H. pylori*, it is not a crucial factor in some geographic regions to determine treatment effectiveness.
10. The table 2, titled "Detection of the cag PAI in *H. pylori* isolates obtained before and after treatment in patients with treatment failure" was deleted and a new table was inserted summarizing the prevalence of *cagA* and *vacA* gene, and the effect of the antibiotic pressure on the virulence-associated genotypes in isolates obtained from 10 Colombian patients with treatment failure.
11. In the results, specifically the section "*Effect of the anti-H.pylori treatment on the number of EPIYA repetitions"* was written again, based on the observations of a reviewer who suggests that the hypothesis of re-infection by new *H. pylori* strains in those patients who showed changes in CagA, it is an explanation is poorly acceptable. About this, we clearly show that the changes in the number of EPIYA repetitions in the *H. pylori* isolates obtained from patients with treatment failure with respect to isolates found before treatment is a effect of the antibiotic pressure on the same isolate (Recrudence), and only in one isolate (SV480 BGC), the change in the number of EPIYA repetitions post-treatment was attributed to the re-infection of this patient with new *H. pylori* strain based on differences in genotyping of isolates and RAPD profiles. Although, the reinfection after unsuccessful eradication is quite uncommon in adults, this may occur and is more likely in developing countries with a high prevalence of *H.pylori* infection. The reinfection rates, in our Nariño population, have been 12% within the first year and 9% each subsequent year.
12. In the discussion, some paragraphs were omitted to make its reading more fluid, reducing its extension. Additionally, the study limitations were discussed taking into account sample size and future directions of the study.
13. The conclusion of the study is clarified.
14. Finally, this manuscript was reviewed for grammatical clarity and appropriate vocabulary by a teacher whose native language is English.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,



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