BAISHIDENG PUBLISHING GROUP INC
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242 Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com http://www.wjgnet.com

Point-by-point responses to reviewer's comments on Manuscript NO.23868

Many thanks for the reviewers' comments. The following are our detailed, point-by-point responses to all the questions raised by the reviewer.

Reviewer reports:

Comments: (Date reviewed: 2016-02-26 18:31)

1. I would like to thank authors for their effort in the manuscript. the manuscript is very well written with correct English. the References are up-to-dated. Although the research on H. pylori was manipulated by many researchers but there are still space for more and more research to be done as long as the disease affects a large sector of population that are facing problems in their treatment as well. Kindly follow my comments within the revised form (made by tracking changes).

   We have made all the revision in the manuscript according to the reviewer's comment.

Comments: (Date reviewed: 2016-03-09 22:01)

1. Moreover, saliva as a source of *Hp*. DNA is questionable. There is a lot of publications corroborating thesis that oral cavity might be the source of *Hp*. stomach infection (which were cited by authors) as those which are in opposition to that……

   Some literatures showed that *H. pylori* infection in the oral cavity and the stomach has a close relation and the mouth is the first extra-gastric reservoir[22, 23], so in this paper we want to analyze the clarithromycin resistance and the mutations consistency between saliva and gastric mutations. We found that the resistance to CLA in gastric mucosa and saliva is quite common, but gastric mucosa and saliva samples might exhibit the different mutation genotypes. Thus, more investigations with a large number of patients are required.

2."More recently, DNA present in saliva has been employed in PCR-based assays designed to detect mutations associated with fragile X syndrome of H. pylori.

   Sorry for our inaccurate description during the text edition. We have revised it. "More recently, DNA present in saliva has been employed in

BAISHIDENG PUBLISHING GROUP INC
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242 Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com http://www.wjgnet.com

PCR-based assays designed to detect mutations associated with fragile X syndrome [34, 35].

Comments: (Date reviewed: 2016-03-10 11:43

1. The authors propose a Nested-ASP-PCR assay to detected *H. pylori* and to evaluate CLA resistance with more sensitivity than ASP-PCR. This is interesting but they must be corrected the following: 1.The Figures 1 and 2 do not show the expected results, described in the text. Specifically, in Fig. 1A the band of 505 bp, which corresponds to the reference strain, cannot be seen. There is only visible a very faint band of approximately 250 bp. In Fig. 1B, the DNA is degraded and the bands in W3 and W4 do not correspond to 294 bp. Please, explain: Why the bands in Fig. 1C are approximately of 400 bp? Furthermore, we observed that the bands in Figures 2B and 2C are very thick and do not correspond to 294 bp.
2. It is necessary that the sensitivity and specificity of Nested-ASP-PCR assay, and also the positive and negative predictive values, might be determinate for both: gastric mucosa and saliva.

We have made the new figures, including Figure 1, 2 and Figure 1, 2 in the supplementary materials.

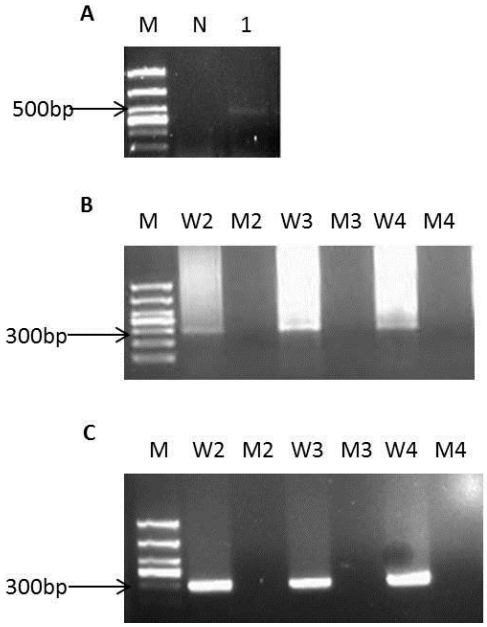

Figure 1 The PCR products of wild-type *Helicobacter pylori* strain in gastric mucosa

A: PCR product with the external primers (M: 1000bp DNA Marker, N: H. pylori negative sample, 1: wild-type strains); B: PCR products with the

different inner primers; C: The products amplified by Nested-ASP-PCR [M: 1000bp DNA Marker (1000, 750, 500, 400, 300, 200, 100), W2: 2142 wild primers (2142A), M2: 2142 mutation primers (2142G), W3: 2143 wild primers (2143A), M3: 2143 mutation primers (2143G), W4: 2144 wild primers (2144A), M4: 2144 mutation primers (2144G)]

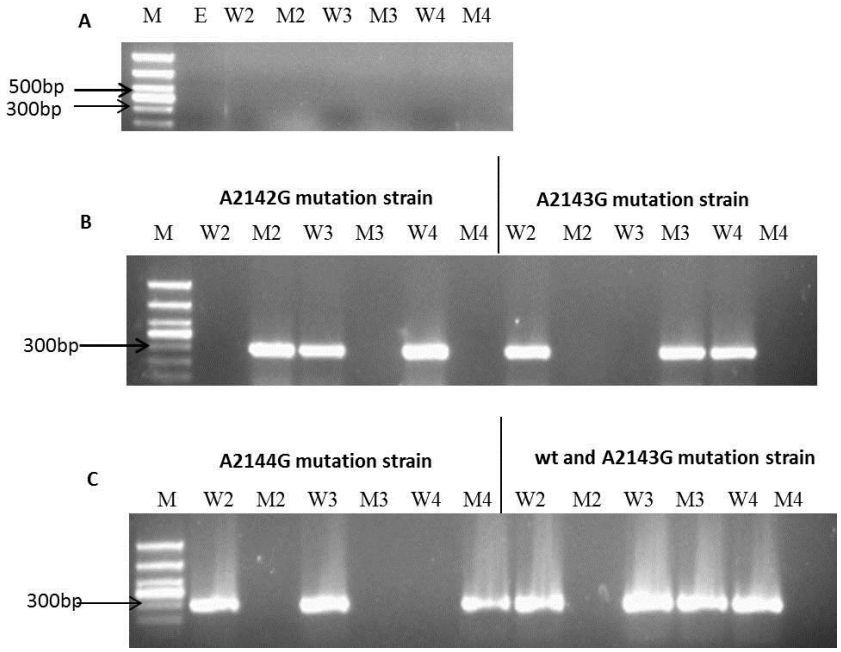

Figure 2 The PCR products of Helicobacter pylori clinical strains in gastric mucosa with 2142, 2143 and 2144 positions mutation assayed by Nested-ASP-PCR

A: *H. pylori* negative control; B: A2142G mutation and A2143G mutation strains; C: A2144G mutation, C: wt and A2143G mutation mixture strains. [M: 1000bp DNA Marker (1000, 750, 500, 400, 300, 200, 100), E: outer PCR primers, W2: 2142 wild primers (2142A), M2: 2142 mutation primers (2142G), W3: 2143 wild primers (2143A), M3: 2143 mutation primers (2143G), W4: 2144 wild primers (2144A), M4: 2144 mutation primers (2144G)]

BAISHIDENG PUBLISHING GROUP INC
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242 Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com http://www.wjgnet.com

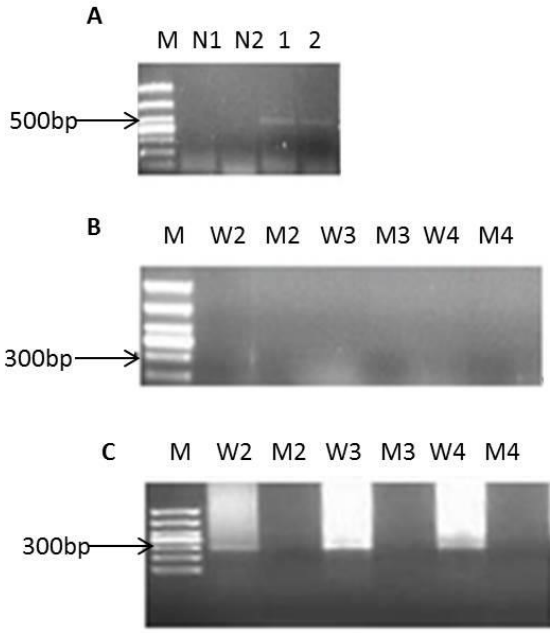

Figure 1(supplementary materials) The PCR products of wild-type *Helicobacter pylori* strain in saliva

A: PCR product with the external primers (M: 1000bp DNA Marker, N1 and N2: *H. pylori* negative sample, 1 and 2: wild-type strains); B: PCR products with the different inner primers; C: The products amplified by Nested-ASP-PCR [M: 1000bp DNA Marker (1000, 750, 500, 400, 300, 200, 100), W2: 2142 wild primers (2142A), M2: 2142 mutation primers (2142G), W3: 2143 wild primers (2143A), M3: 2143 mutation primers (2143G), W4: 2144 wild primers (2144A), M4: 2144 mutation primers (2144G)]

BAISHIDENG PUBLISHING GROUP INC
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242 Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com http://www.wjgnet.com

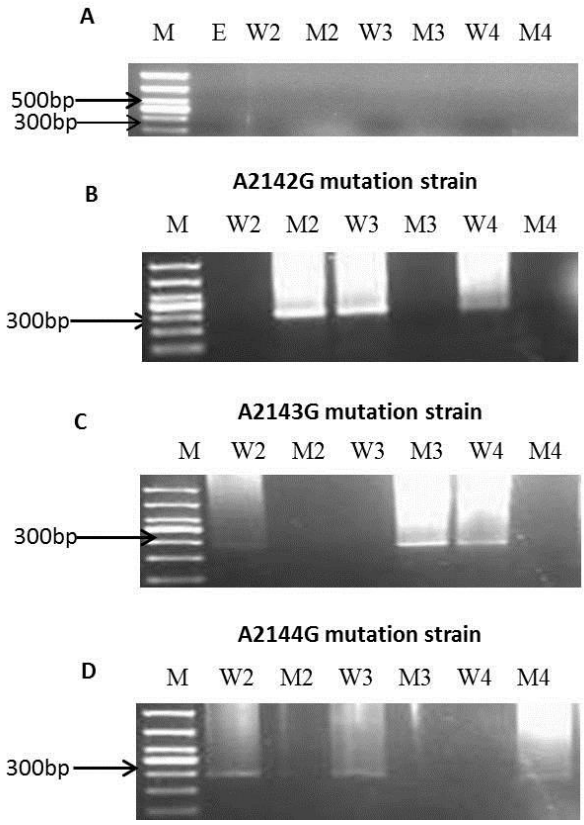

Figure 2(supplementary materials) The PCR products of *Helicobacter pylori* clinical strains in gastric mucosa with 2142, 2143 and 2144 positions mutation assayed by Nested-ASP-PCR

A: *H. pylori* negative control; B: A2142G mutation strain; C: A2143G mutation strain, D: A2144G mutation strain. [M: 1000bp DNA Marker (1000, 750, 500, 400, 300, 200, 100), E: outer PCR primers, W2: 2142 wild primers (2142A), M2: 2142 mutation primers (2142G), W3: 2143 wild primers (2143A), M3: 2143 mutation primers (2143G), W4: 2144 wild primers (2144A), M4: 2144 mutation primers (2144G)]

BAISHIDENG PUBLISHING GROUP INC
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242 Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com http://www.wjgnet.com

Comments: (Date reviewed: 2016-03-20 07:51)

1. In the paper a sensitive procedure (Nested ASP-PCR) is proposed to analyze mutations on *Helicobacter pylori* DNA which confer resistance to Clarithromycin. However, authors should clarify several points in the article, which are described in the file "Comments to authors"

   The mechanism of clarithromycin resistance was stated in second paragraph of "INTRODUCTION" as follows: "Many studies have confirmed that bacterial resistance of *H. pylori* to clarithromycin is associated with the structural change of 23rRNA. This structural change is caused by the single nucleotide polymorphism (SNP) of 23SrRNA [14]. There were A-to-G point mutations at three positions of 2142, 2143 and 2144 within domain V (A2142G, A2143G and A2144G), which have been found to be associated with CLA resistance [2, 15-21]".