

## ESPS PEER-REVIEW REPORT

**Name of journal:** World Journal of Gastroenterology

**ESPS manuscript NO:** 23868

**Title:** Establishment of a nested-ASP-PCR method to determine the clarithromycin resistance of *Helicobacter pylori*

**Reviewer's code:** 03009715

**Reviewer's country:** Egypt

**Science editor:** Jing Yu

**Date sent for review:** 2015-12-31 14:49

**Date reviewed:** 2016-02-26 18:31

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
<input checked="" type="checkbox"/> Grade A: Excellent	<input checked="" type="checkbox"/> Grade A: Priority publishing	Google Search:	<input checked="" type="checkbox"/> Accept
<input type="checkbox"/> Grade B: Very good	<input type="checkbox"/> Grade B: Minor language polishing	<input type="checkbox"/> The same title	<input type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C: Good	<input type="checkbox"/> Grade C: A great deal of language polishing	<input type="checkbox"/> Duplicate publication	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D: Fair	<input type="checkbox"/> Grade D: Rejected	<input checked="" type="checkbox"/> No	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E: Poor		BPG Search:	<input type="checkbox"/> Major revision
		<input type="checkbox"/> The same title	
		<input type="checkbox"/> Duplicate publication	
		<input type="checkbox"/> Plagiarism	
		<input checked="" type="checkbox"/> No	

## COMMENTS TO AUTHORS

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).

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**Name of journal:** World Journal of Gastroenterology

**ESPS manuscript NO:** 23868

**Title:** Establishment of a nested-ASP-PCR method to determine the clarithromycin resistance of *Helicobacter pylori*

**Reviewer's code:** 00183460

**Reviewer's country:** Poland

**Science editor:** Jing Yu

**Date sent for review:** 2015-12-31 14:49

**Date reviewed:** 2016-03-09 22:01

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
<input type="checkbox"/> Grade A: Excellent	<input type="checkbox"/> Grade A: Priority publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B: Very good	<input checked="" type="checkbox"/> Grade B: Minor language polishing	<input type="checkbox"/> The same title	<input type="checkbox"/> High priority for publication
<input checked="" type="checkbox"/> Grade C: Good	<input type="checkbox"/> Grade C: A great deal of language polishing	<input type="checkbox"/> Duplicate publication	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D: Fair	<input type="checkbox"/> Grade D: Rejected	<input type="checkbox"/> Plagiarism	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E: Poor		<input checked="" type="checkbox"/> No	<input type="checkbox"/> Major revision
		BPG Search:	
		<input type="checkbox"/> The same title	
		<input type="checkbox"/> Duplicate publication	
		<input type="checkbox"/> Plagiarism	
		<input checked="" type="checkbox"/> No	

## COMMENTS TO AUTHORS

Authors presented data regarding the development of nested-ASP-PCR methodology to detect SNP in *Helicobacter pylori* 32SrRNA gene, which are suppose to be responsible for CLA resistance. From methodological point of view proposed by authors method is correct. Also the idea behind the experiments seems correct to reduce time of CLA resistance in *Helicobacte* strains isolated from infected patients. Traditional methods involving bacteria culture and E-test or agar diffusion test are really time consuming. However, presented results show that also in case of nested-ASP-PCR full diagnostics of Hp. CLA will involed several reactions. 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 (e.g. 1. Cochrane Database Syst Rev. 2016 Feb 7;2:CD009477. doi: 10.1002/14651858.CD009477.pub2. Periodontal therapy as adjunctive treatment for gastric *Helicobacter pylori* infection. Ren Q1, Yan X, Zhou Y, Li WX. 2. Med Oral Patol Oral Cir Bucal. 2016 Mar 1;21(2):e187-91. Recurrent aphthous stomatitis and *Helicobacter pylori*. Gomes CC1, Gomez RS, Zina LG, Amaral FR. 3. PLoS One. 2015

May 26;10(5):e0126923. doi: 10.1371/journal.pone.0126923. eCollection 2015. Oral and gastric *Helicobacter pylori*: effects and associations. Veiga N1, Pereira C2, Resende C3, Amaral O2, Ferreira M2, Nelas P2, Chaves C2, Duarte J2, Cirnes L3, Machado JC3, Ferreira P4, Correia IJ5. 4. J Physiol Pharmacol. 2004 Jul;55 Suppl 2:105-15. Association of the presence of *Helicobacter pylori* in the oral cavity and in the stomach. Cze?nikiewicz-Guzik M1, Karczewska E, Bielański W, Guzik TJ, Kapera P, Targosz A, Konturek SJ, Loster B. 5. J Physiol Pharmacol. 2006 Sep;57 Suppl 3:91-100. The relationship between the presence of *Helicobacter pylori* in the oral cavity and gastric in the stomach. Loster BW1, Majewski SW, Cze?nikiewicz-Guzik M, Bielanski W, Pierzchalski P, Konturek SJ.) From the literature review it looks like multiplex RT-PCR might be in favor instead of nested-ASP-PCR. Authors should at least try to discuss that possibility. In the "Discussion" section I have found the statement which do not fully understand: "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*." I believe that this must be some kind of general mistake during the text edition. Concluding, in my opinion reviewed paper in its current version does not meet the criteria of publication in World Journal of Gastroenterology.

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**Name of journal:** World Journal of Gastroenterology

**ESPS manuscript NO:** 23868

**Title:** Establishment of a nested-ASP-PCR method to determine the clarithromycin resistance of *Helicobacter pylori*

**Reviewer's code:** 02535852

**Reviewer's country:** Mexico

**Science editor:** Jing Yu

**Date sent for review:** 2015-12-31 14:49

**Date reviewed:** 2016-03-10 11:43

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
<input type="checkbox"/> Grade A: Excellent	<input checked="" type="checkbox"/> Grade A: Priority publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B: Very good	<input type="checkbox"/> Grade B: Minor language polishing	<input type="checkbox"/> The same title	<input type="checkbox"/> High priority for publication
<input checked="" type="checkbox"/> Grade C: Good	<input type="checkbox"/> Grade C: A great deal of language polishing	<input type="checkbox"/> Duplicate publication	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D: Fair	<input type="checkbox"/> Grade D: Rejected	<input type="checkbox"/> Plagiarism	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E: Poor		<input checked="" type="checkbox"/> No	<input checked="" type="checkbox"/> Major revision
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		<input type="checkbox"/> Duplicate publication	
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		<input checked="" type="checkbox"/> No	

## COMMENTS TO AUTHORS

The authors propose a Nested-ASP-PCR assay to detect *H. pylori* and to evaluate CLA resistance with more sensitivity than ASP-PCR. This is interesting but they must be corrected the following:

- 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.
- 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.

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**Name of journal:** World Journal of Gastroenterology

**ESPS manuscript NO:** 23868

**Title:** Establishment of a nested-ASP-PCR method to determine the clarithromycin resistance of *Helicobacter pylori*

**Reviewer's code:** 03010068

**Reviewer's country:** Argentina

**Science editor:** Jing Yu

**Date sent for review:** 2015-12-31 14:49

**Date reviewed:** 2016-03-20 07:51

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
<input type="checkbox"/> Grade A: Excellent	<input type="checkbox"/> Grade A: Priority publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B: Very good	<input type="checkbox"/> Grade B: Minor language polishing	<input type="checkbox"/> The same title	<input type="checkbox"/> High priority for publication
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		BPG Search:	<input checked="" type="checkbox"/> Major revision
		<input type="checkbox"/> The same title	
		<input type="checkbox"/> Duplicate publication	
		<input type="checkbox"/> Plagiarism	
		<input type="checkbox"/> No	

## COMMENTS TO AUTHORS

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".